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Search for What's Missing: Unknown Compound Characterization Using LC-MS

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Small Molecule Structure Analysis

Small molecule structure analysis encompasses broad applications:

Pharmaceutical, metabolomics, food & environmental, clinical, forensic, industrial chemical, etc.

The following are small molecule structure analyses and are crucial for pharma R&D.

- Impurity analysis
  - Drug substance (API)
  - Drug product
  - Genotoxic
  - Stability studies - degradants
- Met ID
  - Drug discovery – pre-clinical development – clinical development
  - *In vitro* (hepatocytes, microsomes, whole blood, plasma)
  - *In vivo* (whole blood, plasma, urine, bile, fecal homogenates)
  - Radio-labeled (^{13}C, ^{14}C, ^{3}H)
- E&L analysis
  - Extractables
  - Leachables
- Natural products & traditional medicines research
  - Discovery and Identify new medicine

Small Molecule Structure Analyses are Challenging and Complex

- Broad range of chemicals with very diverse structures
  - ChemSpider has 71 M chemical structures.
- Background Interference
  - Complex biologic matrices, excipients, and solvent background
- Sample Limitations
  - Example: ADC drug Met ID, the small molecule “warhead” is only small portion of the drug.
- Unknowns
  - *De Novo* structural determination is not trivial!
- Many small molecule structure analyses are highly regulated.
  - Pharma R&D must follow EPA, FDA, EMEA, countries' regulations and guidelines: compliance and GLP.
  - Toxicity assessments, clinic trails... to ensure drug efficacy and consumer safety

All decisions must be based on solid scientific analysis results.

High Quality Data is Vital!

Reference: Mass Spectrometry Identification Categories in USP Chapter <1663>

Data typically available from GC/MS and LC/MS analyses (see A through E below) are used to designate individual extractables identifications in the categories of ***Confirmed, Confident***, or ***Tentative***

A. Mass spectrometric fragmentation behavior

B. Confirmation of molecular weight

C. Confirmation of elemental composition

D. Mass spectrum matches automated library or literature spectrum

E. Mass spectrum and chromatographic retention index match authentic reference compound

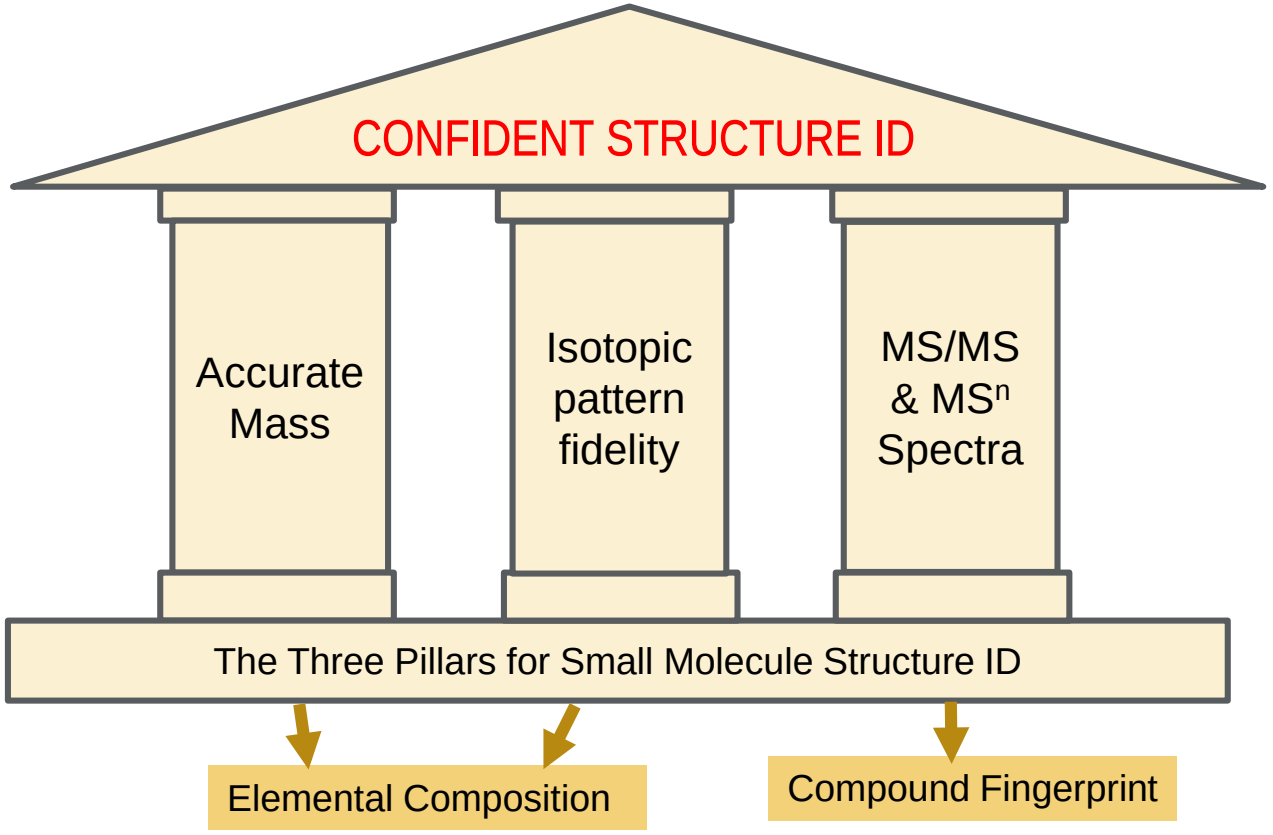
Confirmed - A ***Confirmed*** identification means that A, B (or C), and D (or E) have been fulfilled.

Confident - A ***Confident*** identification means that sufficient data to preclude all but the most closely related structures have been obtained. The combination of D with any of A, B, or C can be used to provide a confident identification

Tentative - A ***Tentative*** identification means that data have been obtained that are consistent with a class of molecule only.

Confident Structure ID Requires High Resolution Accurate Mass (HRAM) and MS^n Fragments

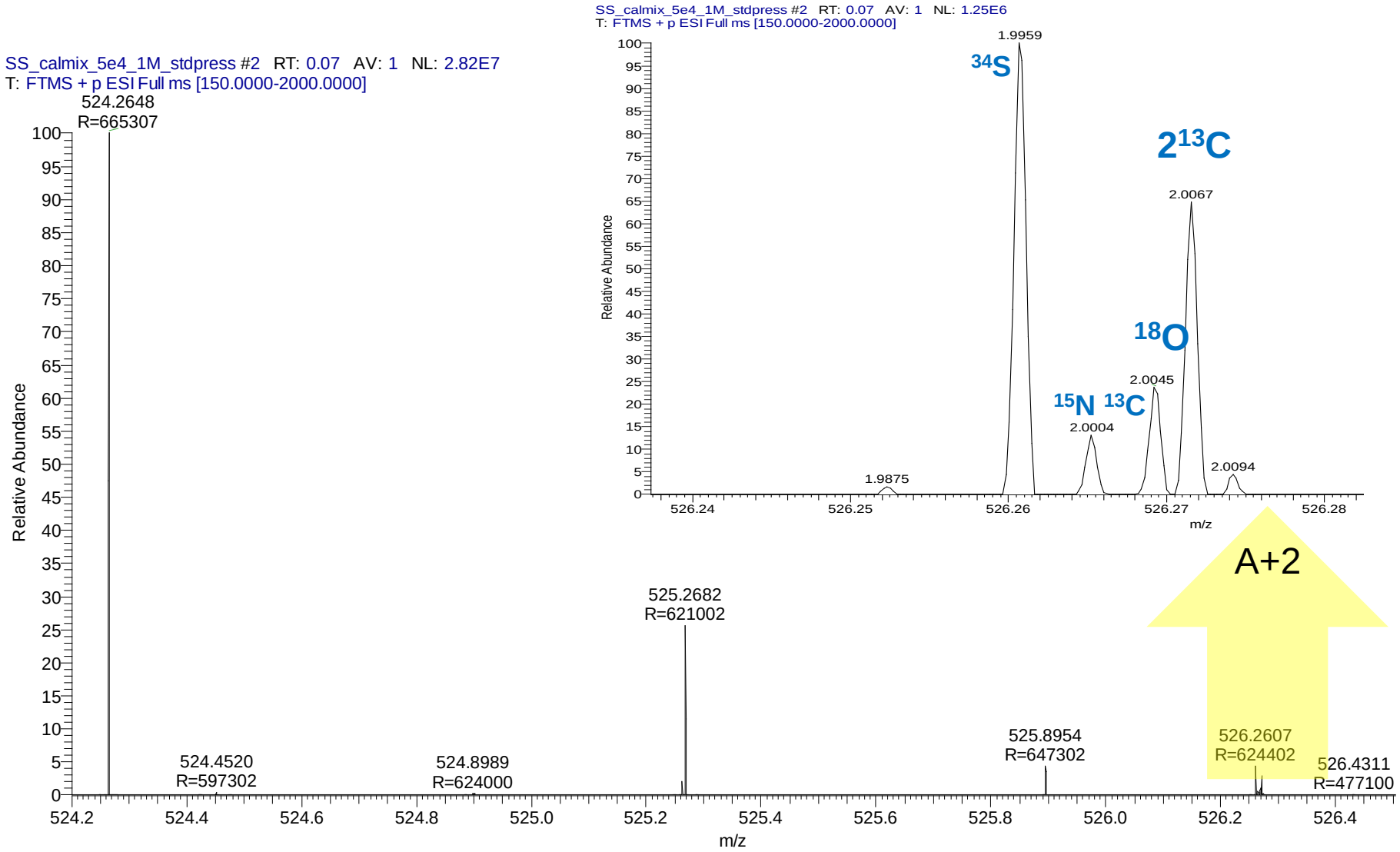
Small Molecule Structure Identification



The Instrument of Choice: Orbitrap™ Mass Spectrometer

The Power of High Resolution MS: Fine Isotope Structure MRFA

MRFA



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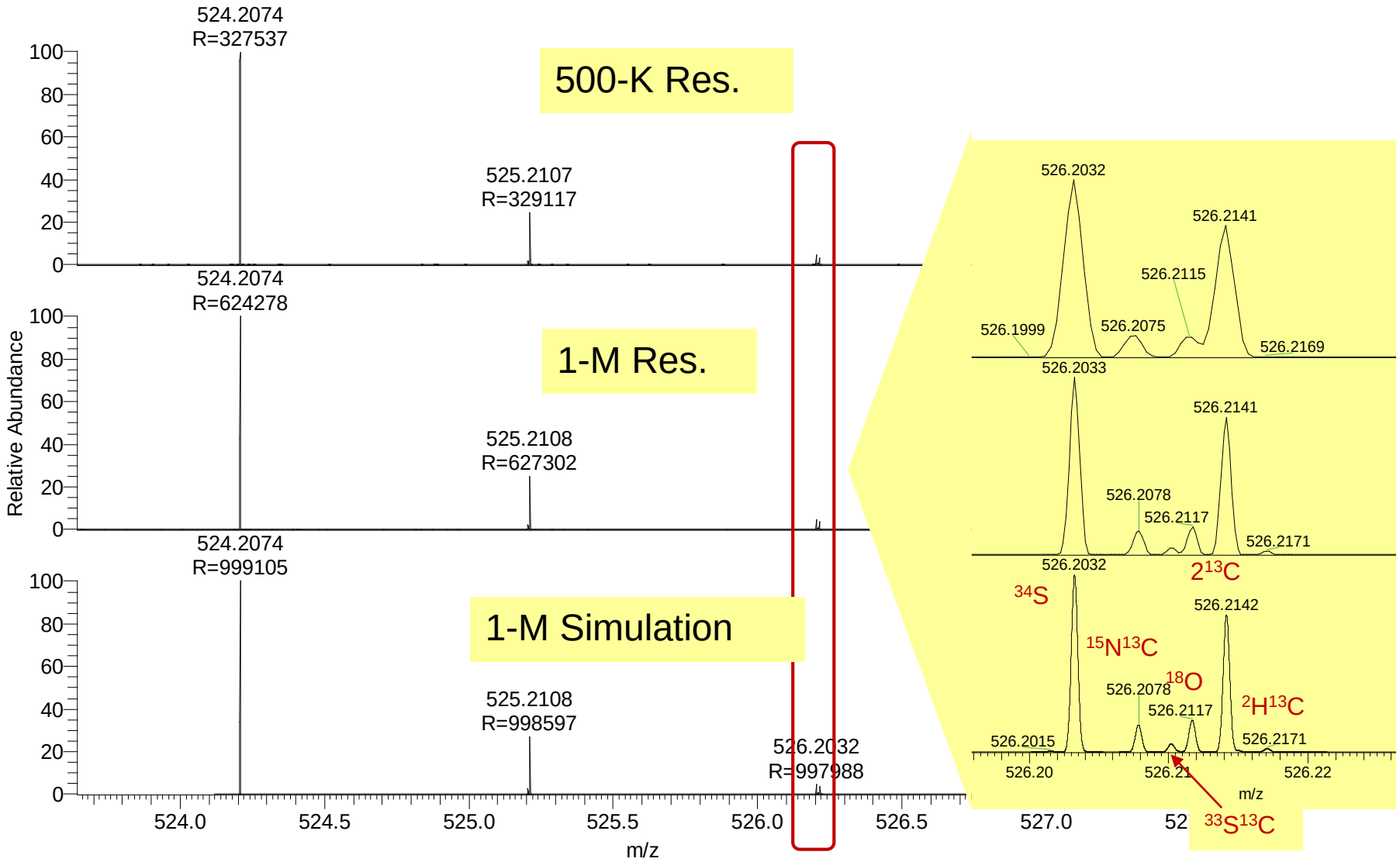
Ultra High Resolution for Unknown Impurity ID



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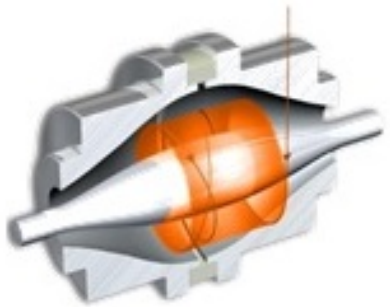
Orbitrap MS for Small Molecule Structure Analysis

Thermo Scientific LTQ Orbitrap MS – First Generation Hybrid MS



Since 2005 ASMS introduction, Orbitrap MS has become the gold standard for small molecular structure analysis.

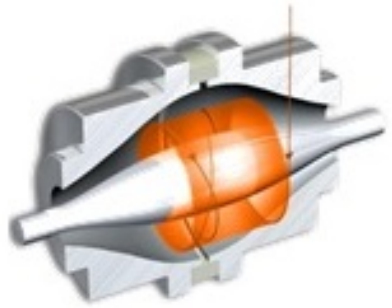
Thermo Scientific Q Exactive MS Family – Quan/Qual Workhorse

Thermo Scientific™
Q Exactive™ HF MS

Resolving Power: 240K @ m/z 200
Scan Range: 50-6,000
Scan rate: 18Hz at 15K

Thermo Scientific™
Q Exactive™ Plus MS

Resolving Power: 140K @ m/z 200
Scan Range: 50-6,000
Scan Rate: 12 Hz at 17.5K
Optional: 280K



Thermo Scientific™
Q Exactive ™ MS

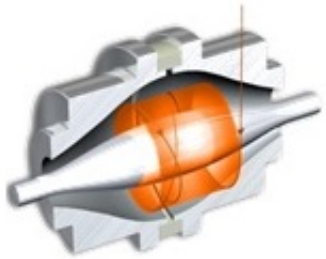
Resolving Power: 140K @ m/z 200
Scan Range: 50-6,000
Scan Rate: 12 Hz at 17.5K

Thermo Scientific™ Q Exactive™ Focus MS

Resolving Power: 70K @ m/z 200
Scan Range: m/z 50-2,000
Scan Rate: 12 Hz at 17.5K

First bench-top Orbitrap MS
High performance
Easy to use, robust
Polarity switching <1 sec
HCD MS2

Transforming Small Molecule Identification and Characterization



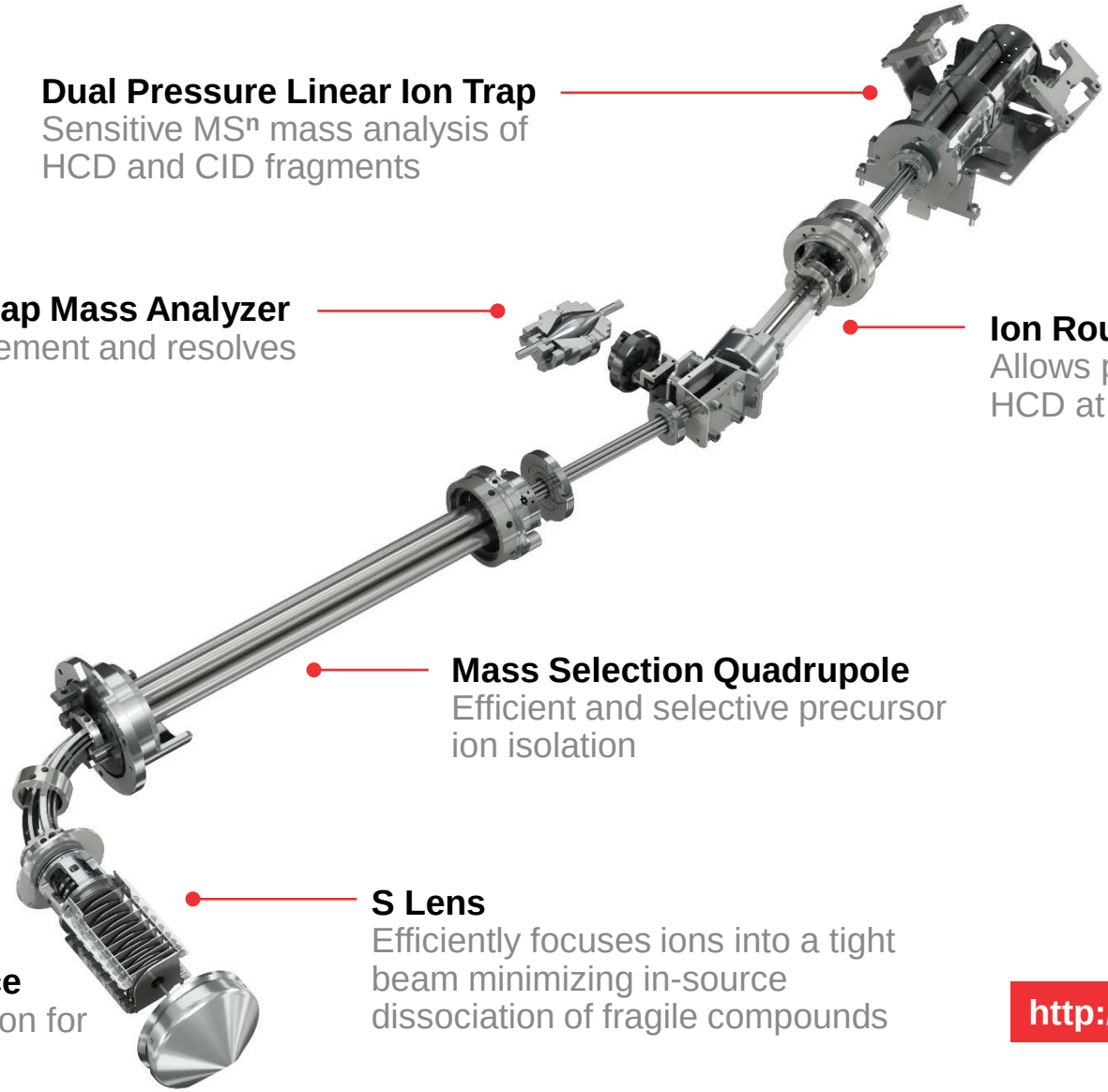
- Mass Range *m/z* 50 – 2000
- Mass Accuracy <1ppm internal, <3ppm external
- Max. Mass Resolution >500,000
- Scan rate 30 Hz OT MS^2 /40 Hz IT MS^2
- Dissociation HCD, CID
- MS/MS and MS^n
- Polarity switching on the fly

Thermo Scientific™ Orbitrap ID-X™ Tribrid™ MS – Optimized and dedicated to small molecule structure analysis

- High performance
  - Very high resolution (500K at m/z 200) and high scan rate (30 Hz OT/40 Hz IT)
  - MS^n, CID/HCD multiple dissociation techniques in a single run, OT/IT parallel detection …
- Ultimate flexibility and capability for data acquisition
  - Comprehensive, feature-specific filters enable triggering MS^n of low abundant components
  - Predefined method templates for quick start
- Novel data acquisition – AcquireX
  - Automatic background exclusion, greatly improves efficiency, quality and accuracy of analysis
- Advanced data processing SW suite and database
  - Thermo Scientific™ Compound Discoverer™ 3.0 software, Thermo Scientific™ Mass Frontier™ 8.0 software, and mzCloud™.

mzCloud is a trademark of HighChem LLC, Slovakia

Schematic for Thermo Scientific Orbitrap ID-X MS – Improved Instrumentation



Dual Pressure Linear Ion Trap
Sensitive MS^n mass analysis of HCD and CID fragments

Ultra High Field Orbitrap Mass Analyzer
Accurate mass measurement and resolves isobaric species

Ion Routing Multipole
Allows parallel analysis HCD at any fragmentation level

Mass Selection Quadrupole
Efficient and selective precursor ion isolation

Active Beam Guide
Prevents neutrals and high velocity clusters from entering mass selecting quadrupole

S Lens
Efficiently focuses ions into a tight beam minimizing in-source dissociation of fragile compounds

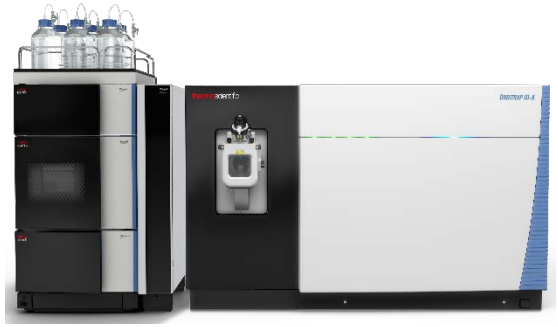
Optional Easy-IC Ion Source
Generates internal calibrant ion for real-time mass calibration

http://planetorbitrap.com

General Workflow for Small Molecule Structure Analysis

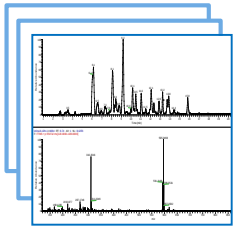
Metabolite ID

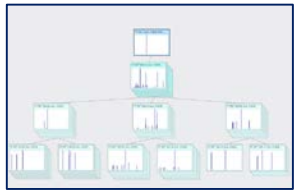
Orbitrap ID-X MS



AcquireX Data Acquisition

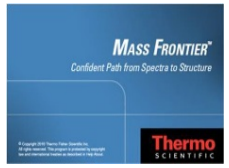
High Quality HRAM MS & MS^n





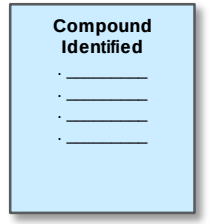
Novel Software & Database

Compound Discoverer 3.0

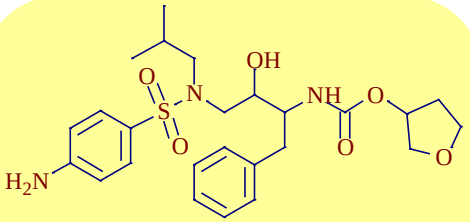


Mass Frontier 8.0

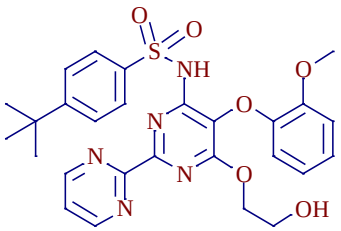
Confident Result



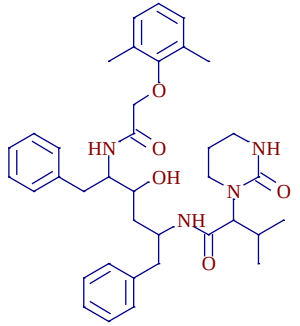
Case Study: Metabolite Identification of 5 Drugs in Human Liver Microsomal Incubation



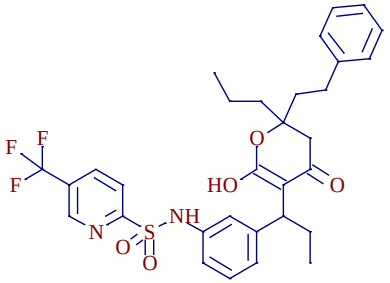
Amprenavir
C25H35N3O6S
$(M+H)^+$ 506.23193
Cas# 161814-49-9



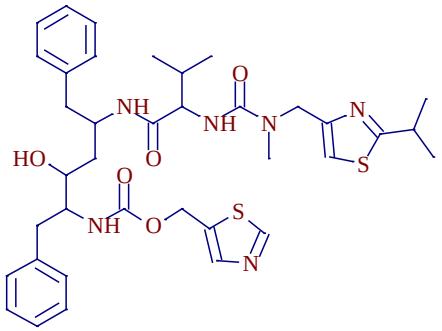
Bosentan
C27H29N5O6S
$(M+H)^+$ 552.19113
Cas# 147536-97-8



Lopinavir
C37H48N4O5
$(M+H)^+$ 629.36975
Cas# 192725-17-0



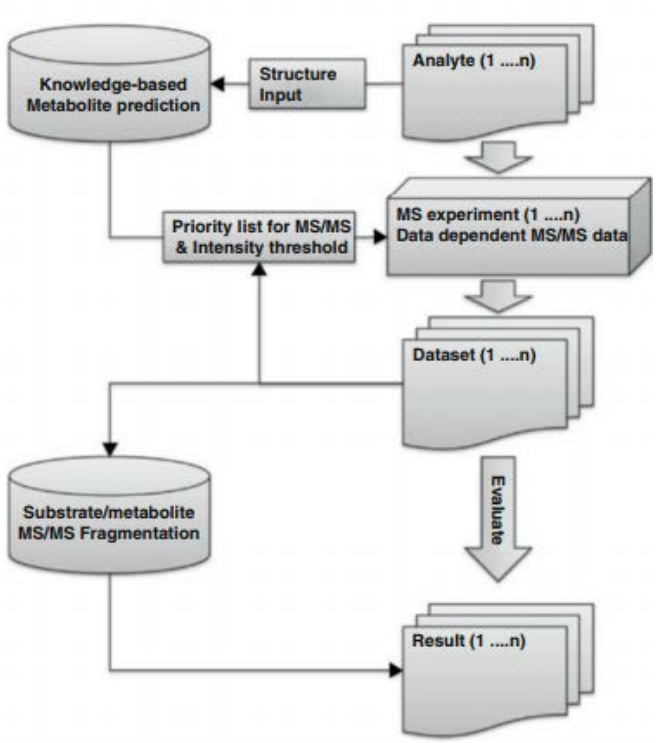
Tipranavir
C31H33F3N2O5S
$(M+H)^+$ 603.21350
Cas# 174484-41-4



Ritonavir
C37H48N6O5S2
$(M+H)^+$ 721.32004
Cas# 155213-67-5

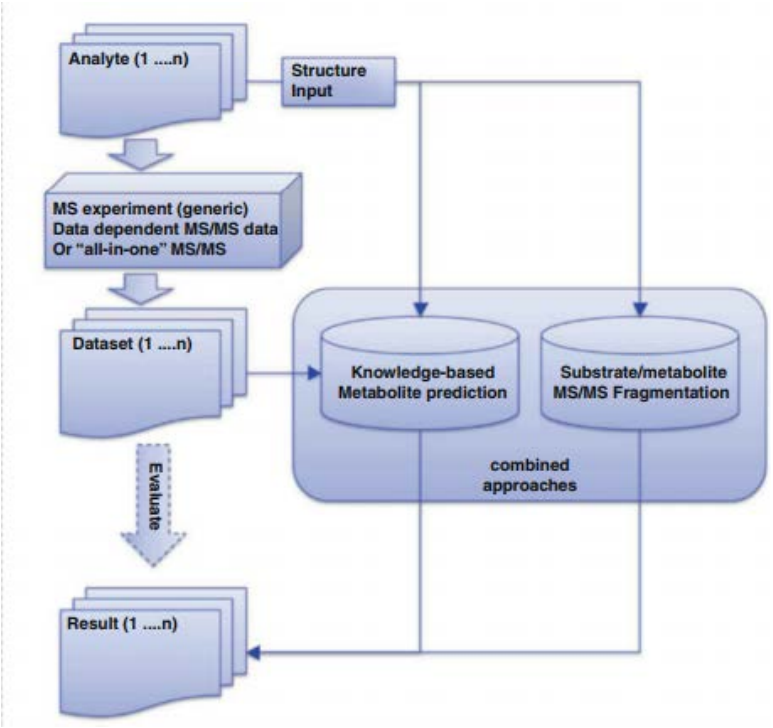
Traditional Data Dependent Acquisition of MS/MS Spectra for Drug Metabolites

Targeted Approach



DDA Selection Criteria:
- m/z inclusion list based on predicted metabolites
- Isotope pattern
- Mass defect

Non-targeted Approach



DDA Selection Criteria:
- Intensity based ("top N" most intense)
- All ion fragmentation

Pähler and Brink, *Drug Discov Today Technol.,* **2013**, 10, e207-213

AcquireX with Orbitrap ID-X MS – Tackles the Identification Bottleneck Automatically

- Automatically generates an exclusion list from a control sample.
  - Excludes the background ions from triggering MS^n
  - Intelligent data-dependent acquisition - only triggers the ions of interest that are not present in the control.
- Automatically generate an inclusion list of samples for deep scan when needed.
- High quality MS^n data in one run and no repeat injections, no need for user to build exclusion and inclusion list offline
- Acquire useful data - better than data-independent acquisition (DIA)

AcquireX greatly improves analysis efficiency, quality, and accuracy!

AcquireX Acquisition Workflow: High Performance and Easy to Use

Predefined Method Templates for Met ID

AcquireX Acquisition Workflow

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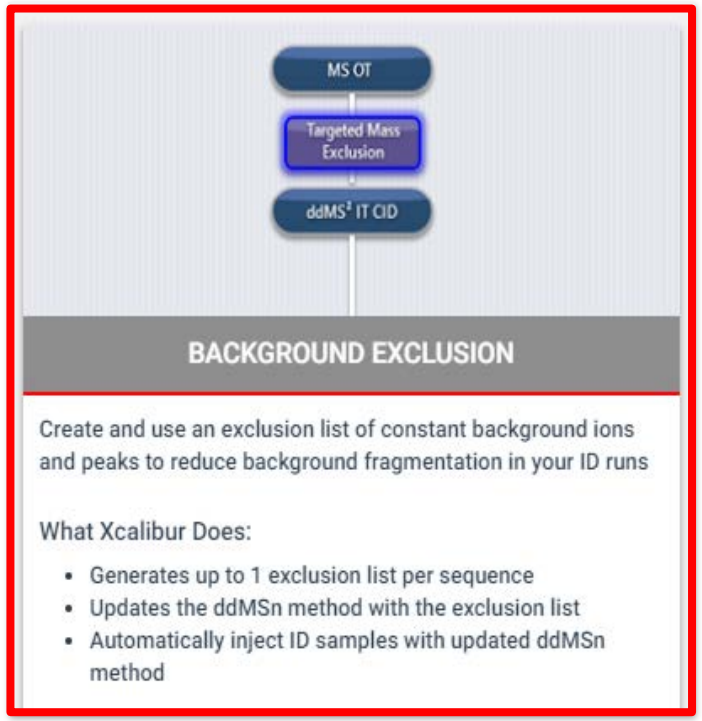
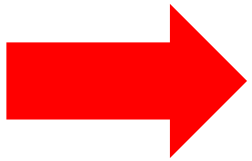
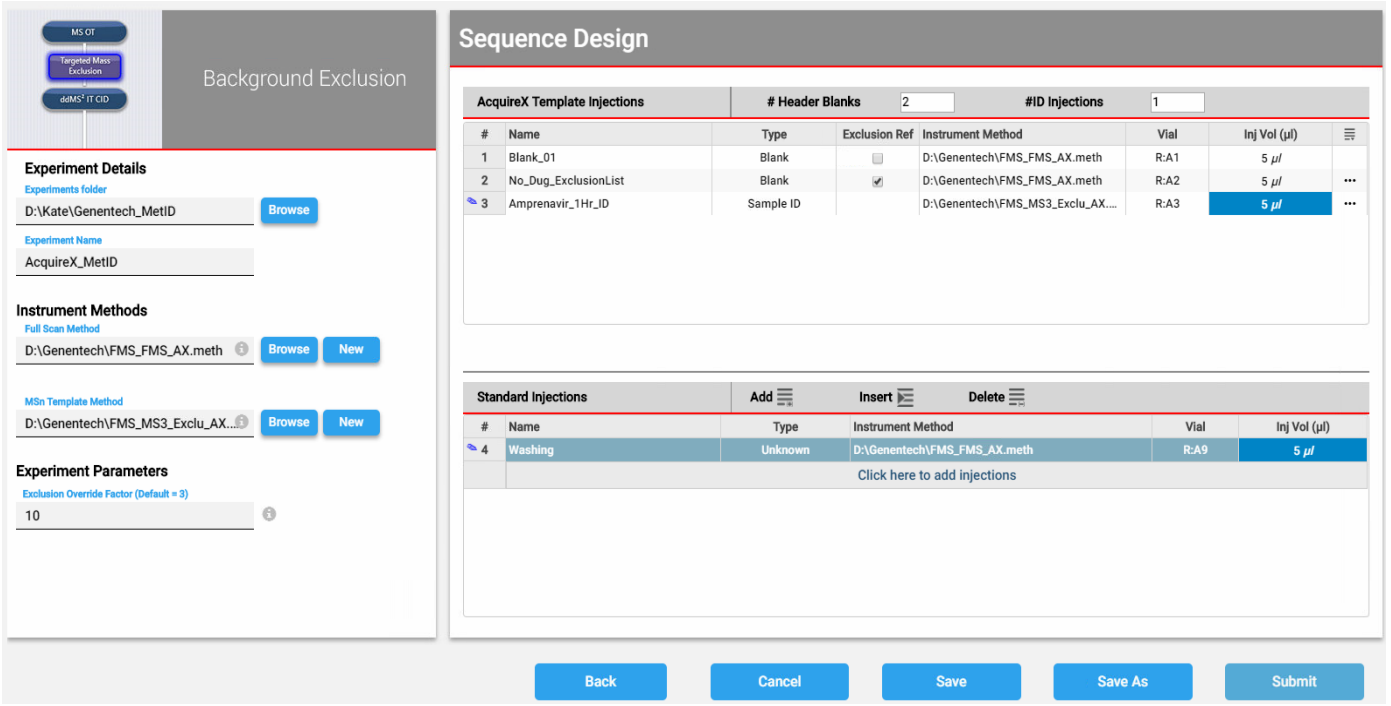
AcquireX Data Acquisition Workflow and Sequence Setup







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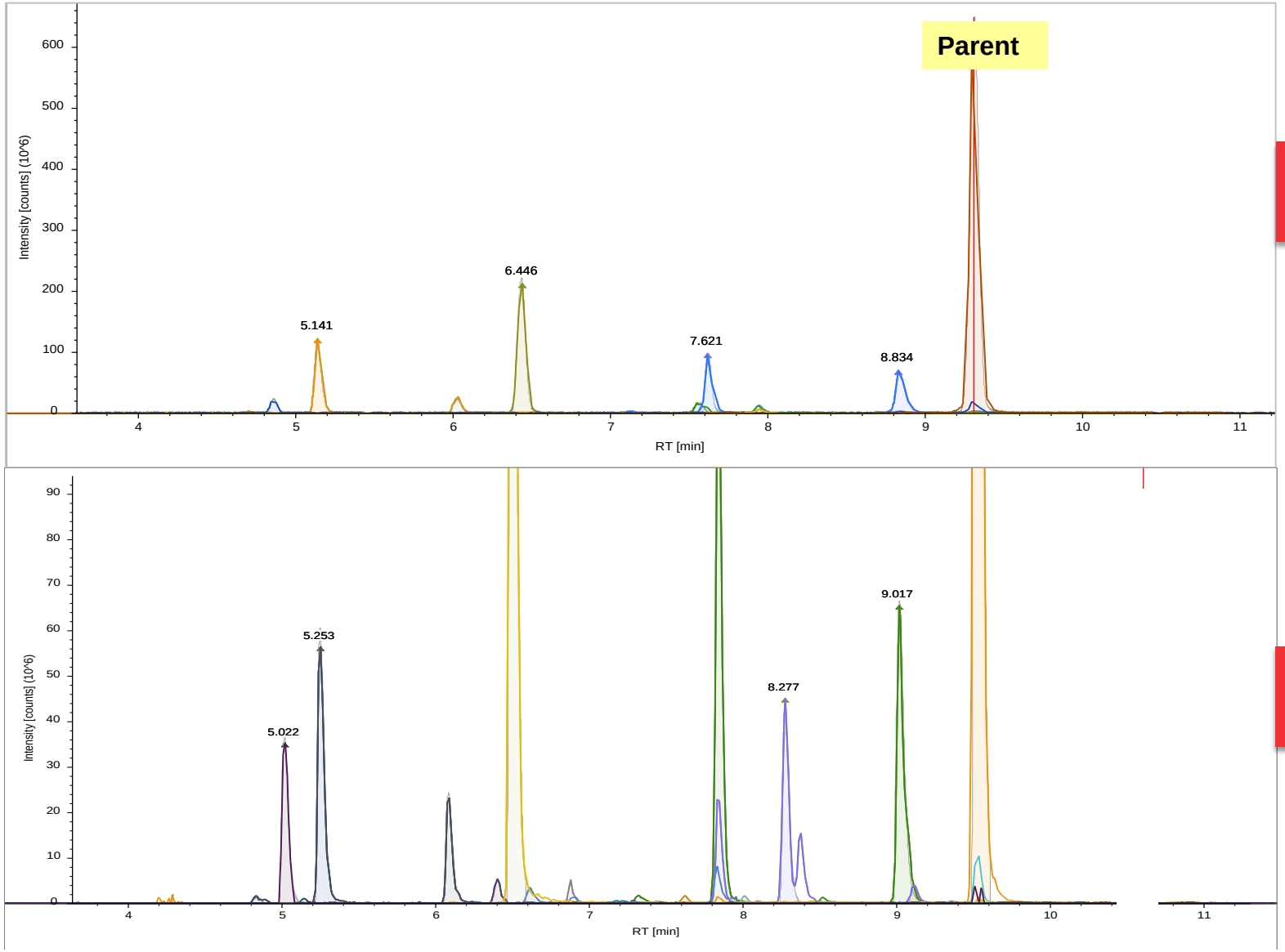
Conventional and AcquireX Workflow Comparison: Amprenavir as an Example



DDA triggered 8 metabolites

AcquireX triggered 21 metabolites

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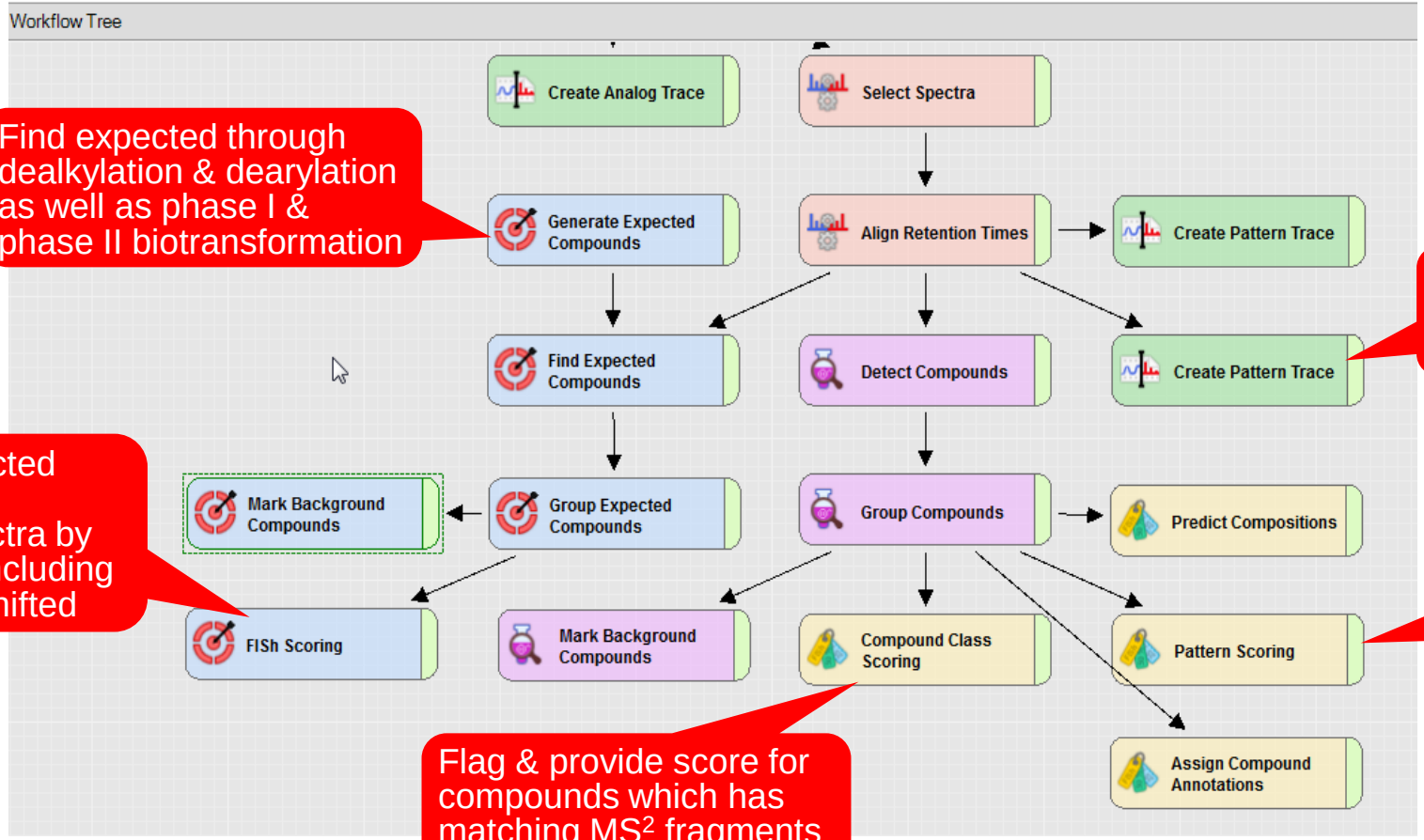
Data Processing Using Thermo Scientific Compound Discoverer 3.0 (CD 3.0) Software

Utilizing unique features of CD 3.0, metabolites both expected and unknown were identified using pre-made “Expected and Unknown” processing workflow



Find expected through dealkylation & dearylation as well as phase I & phase II biotransformation

XIC traces for analytes containing user-defined isotopic patterns

Provide score for detected expected compounds; annotates on MS^n spectra by fragment ion search, including transformation mass shifted

Provide score for analytes containing user-defined isotopic patterns

Flag & provide score for compounds which has matching MS^2 fragments with parent compound

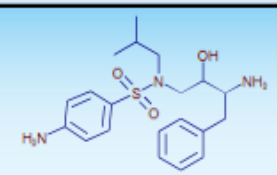
“Generate Expected Compounds” Prediction in CD 3.0

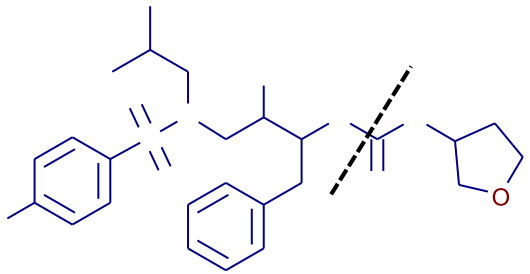
Parameters of 'Generate Expected Compounds'

Show Advanced Parameters

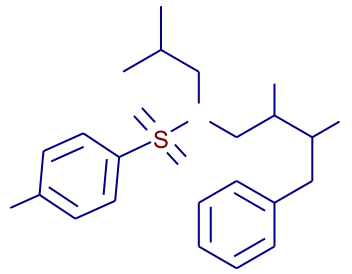
1. Compound Selection	
Compound	161814-49-9 Amprenavir (C25 H35 N3 O6 S)
2. Dealkylation	
Apply Dealkylation	True
Apply Dearylation	True
Max. # Steps	2
Min. Mass [Da]	150
3. Transformations	
Phase I	Dehydration (H2 O ->); Desaturation (H2 ->); Hyc
Phase II	Acetylation (H -> C2 H3 O); Arginine Conjugation
Others	
Max. # Phase II	1
Max. # All Steps	3
4. Ionization	
Ions	[M+H]+1; [M-H]-1

Expected Compounds | Expected Formulas | Expected Features | Related Structures | Input Files

	Checked	Parent Compound	Formula	Molecular Weight	Dealkylated	Composition Cha	Structure
1	☐	Amprenavir	C20 H29 N3 O3 S	391.19296	X	-(C5 H6 O3)	



Hydrolysis

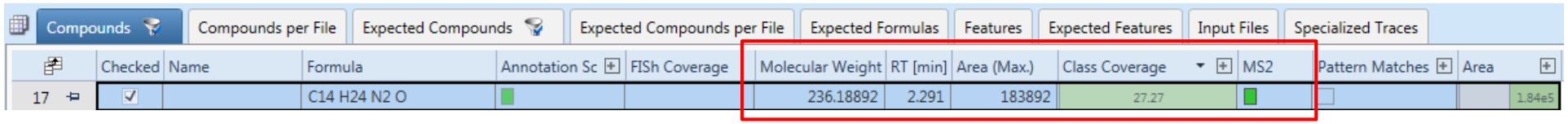


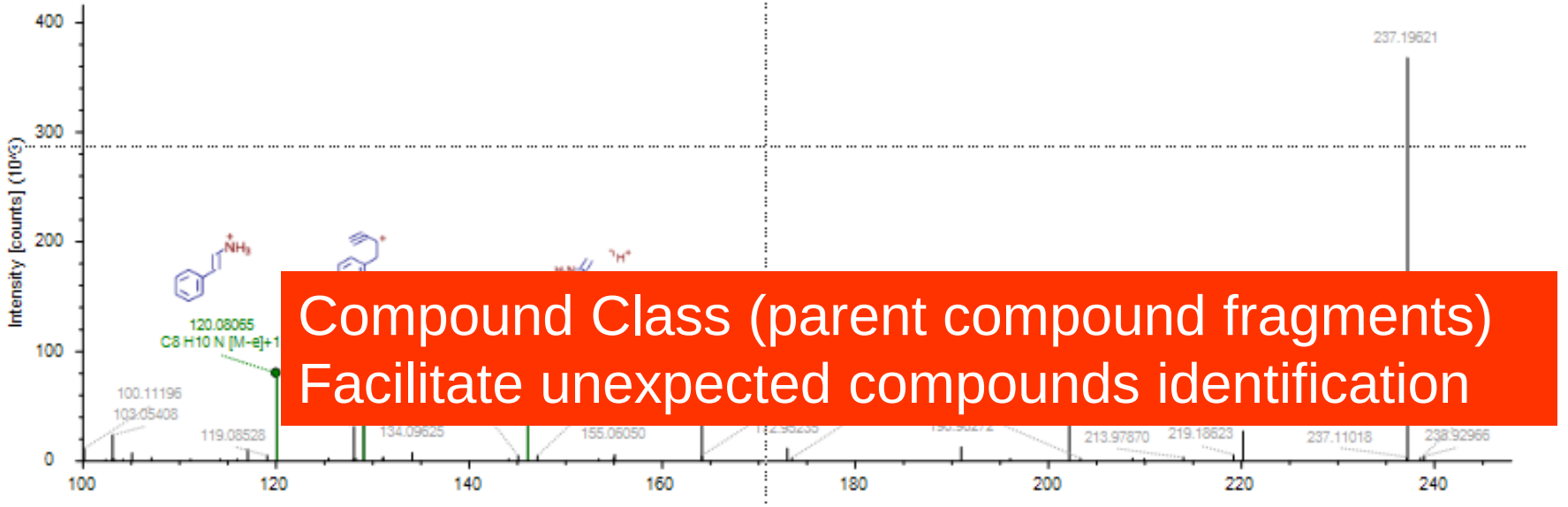
parent 506.2320 @9.52min

392.2002 @6.50min

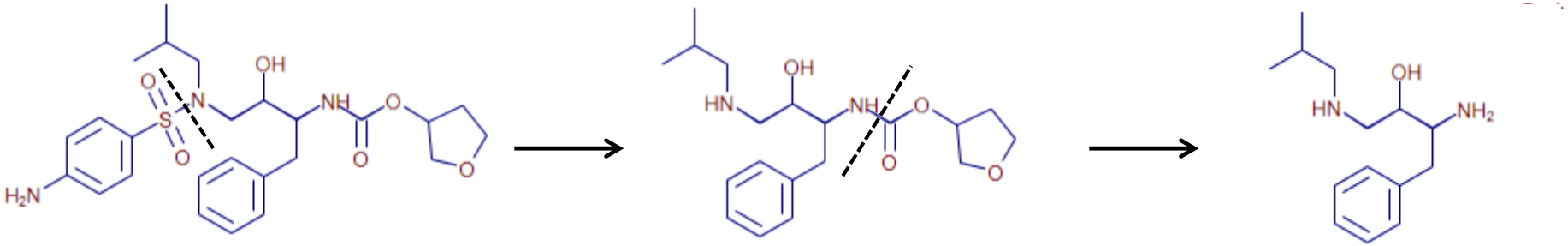
Dealkylation and Dearylation followed Phase I, Phase II transformation better prediction for expected compounds

Compound Class for Unexpected Metabolite Identification





Compound Class (parent compound fragments)
Facilitate unexpected compounds identification



m/z 506.2319 m/z 351.2278 m/z 237.1961

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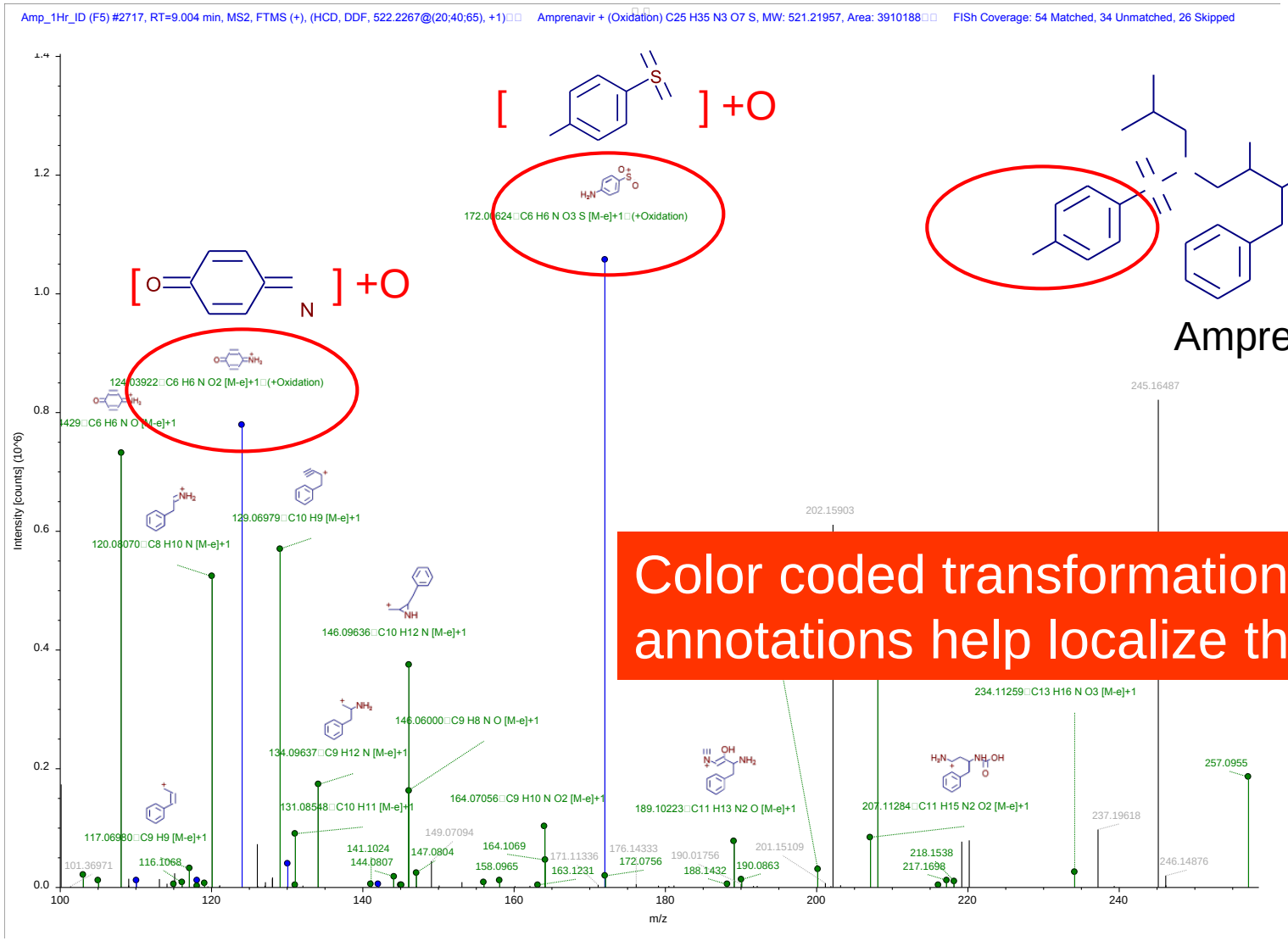
Fragment Ion Search (FISh) Annotations

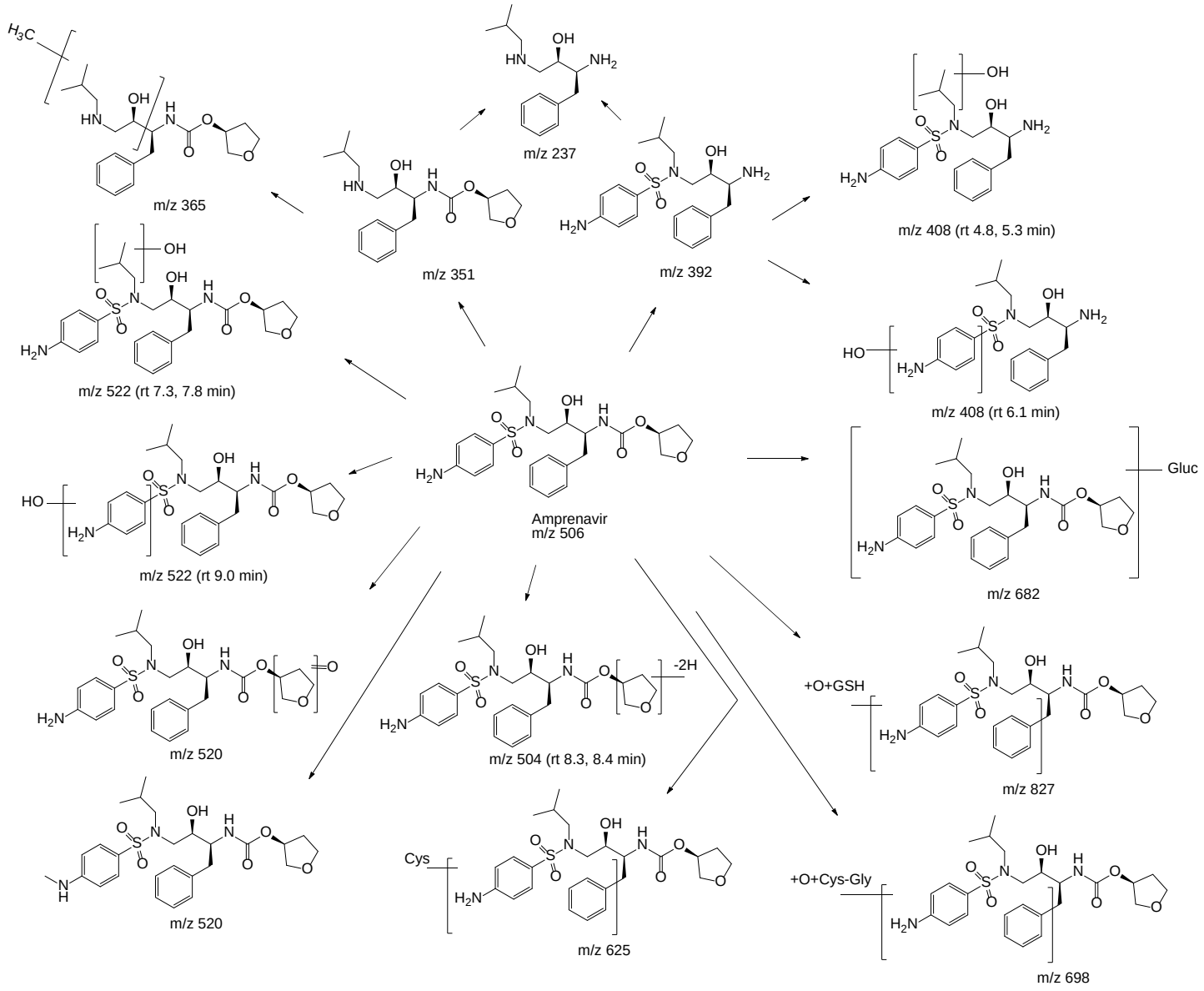


Color coded transformation shifted fragment ion annotations help localize the site of biotransformation.

More Metabolites were Triggered for MS^n Using Acquire X than Conventional DDA

RT [min]	Molecular Weight	Formula	Transformations	DDA	AcquireX
2.29	236.1889	C14H24N2O	Sulfonamide hydrolysis + amide hydrolysis		Y
4.84	407.18788	C20H29N3O4S	Amide hydrolysis + oxidation		Y
5.02	350.22056	C19H30N2O4	Sulfonamide hydrolysis	Y	Y
5.14	364.23621	C20H32N2O4	Sulfonamide hydrolysis + methylation		Y
5.25	407.18788	C20H29N3O4S	Amide hydrolysis + oxidation	Y	Y
6.09	407.18788	C20H29N3O4S	Amide hydrolysis + oxidation	Y	Y
6.41	697.24513	C30H43N5O10S2	Oxidation + Cys-Gly-conjugation		Y
6.50	391.19296	C20H29N3O3S	Amide hydrolysis	Y	Y
6.61	519.20392	C25H33N3O7S	Oxidation (+O-2H)	Y	Y
6.88	826.28773	C35H50N6O13S2	Oxidation + GSH Conjugation		Y
7.19	624.22876	C28H40N4O8S2	Cysteine Conjugation		Y
7.32	521.21957	C25H35N3O7S	Oxidation	Y	Y
7.62	405.20861	C21H31N3O3S	Amide hydrolysis + methylation		Y
7.81	681.25674	C31H43N3O12S	Glucuronidation	y	Y
7.84	521.21957	C25H35N3O7S	Oxidation	Y	Y
8.01	537.21449	C25H35N3O8S	Di-oxidation		Y
8.28	503.20901	C25H33N3O6S	Dehydration		Y
8.37	503.20901	C25H33N3O6S	Dehydration		Y
9.02	521.21957	C25H35N3O7S	Oxidation		Y
9.11	503.20901	C25H33N3O6S	Dehydration		Y
9.52	505.2246	C25H35N3O6S	**Amprenavir Parent**	Y	Y
10.61	519.24031	C26H37N3O6S	Methylation		Y

Metabolites Identified from Amprenavir HLM Incubations



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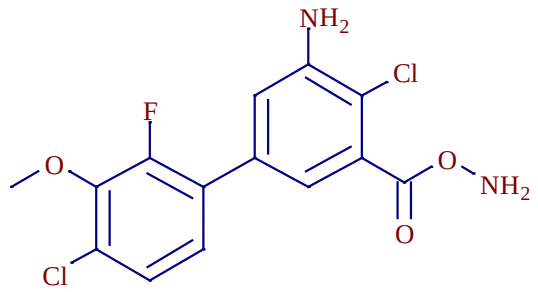
Isotope Triggered Trace Level Metabolite MS^n

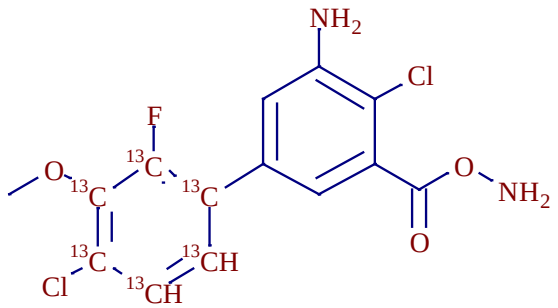
Use of Isotopically Enhanced Labels for Plant Metabolite Identification

A

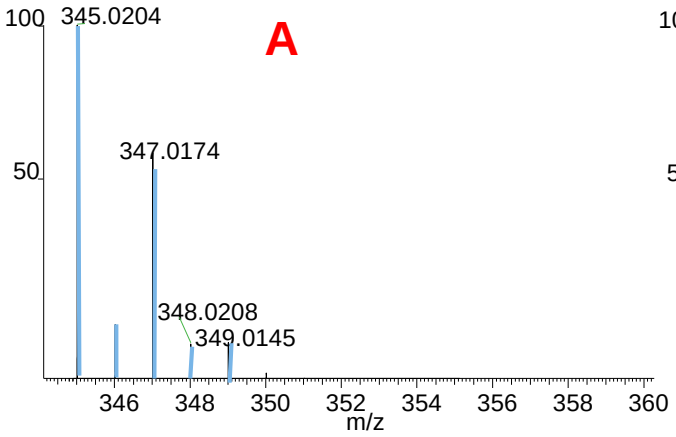


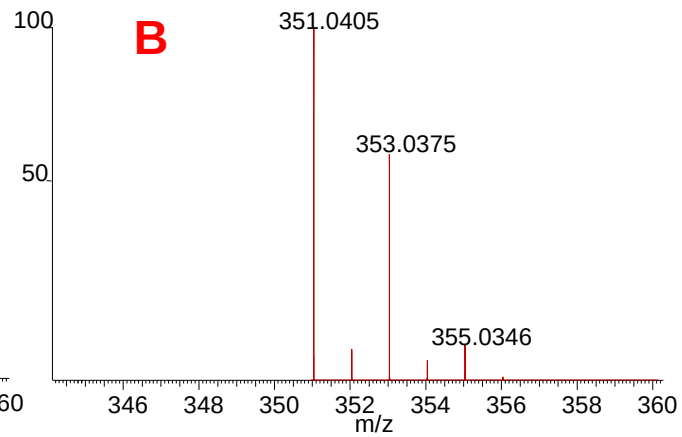
$C_{14}H_{11}Cl_2FN_2O_3$
Mass 344.01308
$(M+H)^+$ 345.02035
Cas# 943831-98-9

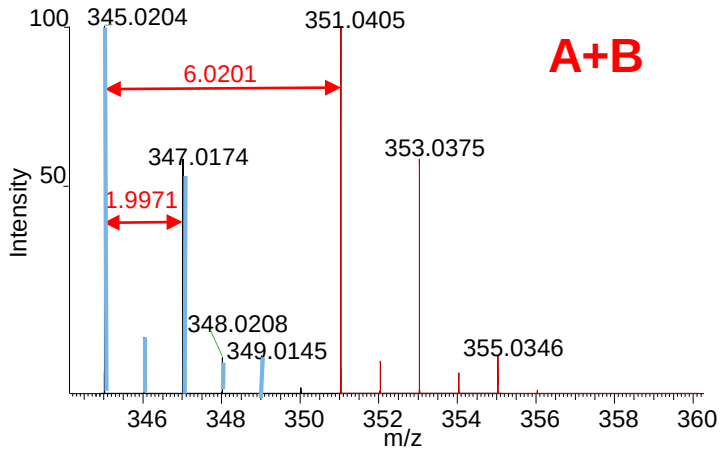
B



$C_8{}^{13}C_6H_{11}Cl_2FN_2O_3$
Mass 350.03320
$(M+H)^+$ 351.04048



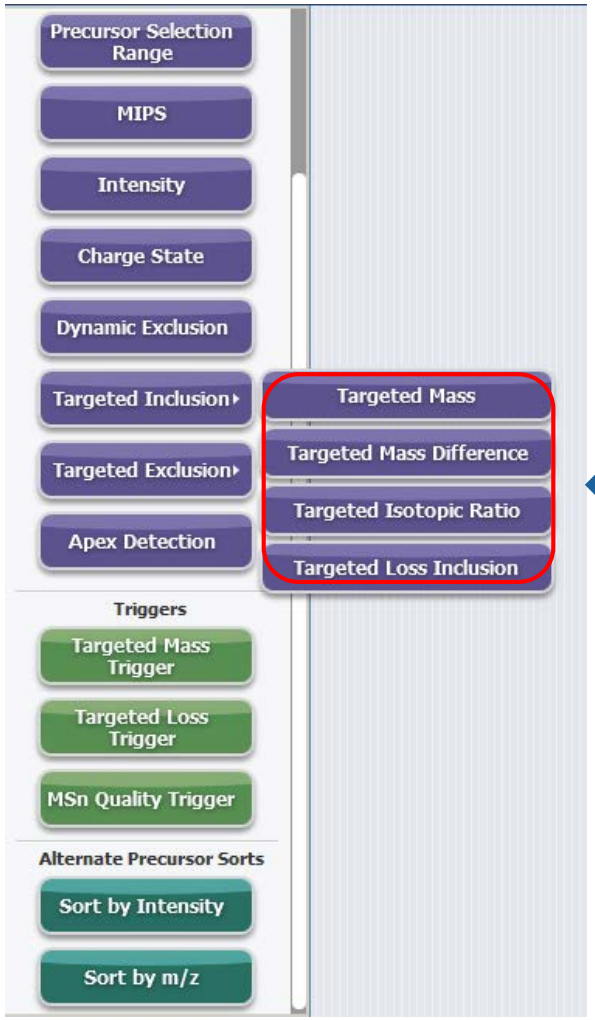




Blend of A and B yields significant +2, +6, +8 isotopic pattern

ThermoFisher SCIENTIFIC

Feature-Specific Filters for Trace Level Labeled Metabolite ID



The feature-specific filters in ID-X method editor allow user to build sophisticated instrument method to capture trace level metabolites present in complex matrices, triggering MS^n for confident identification and structure elucidation.

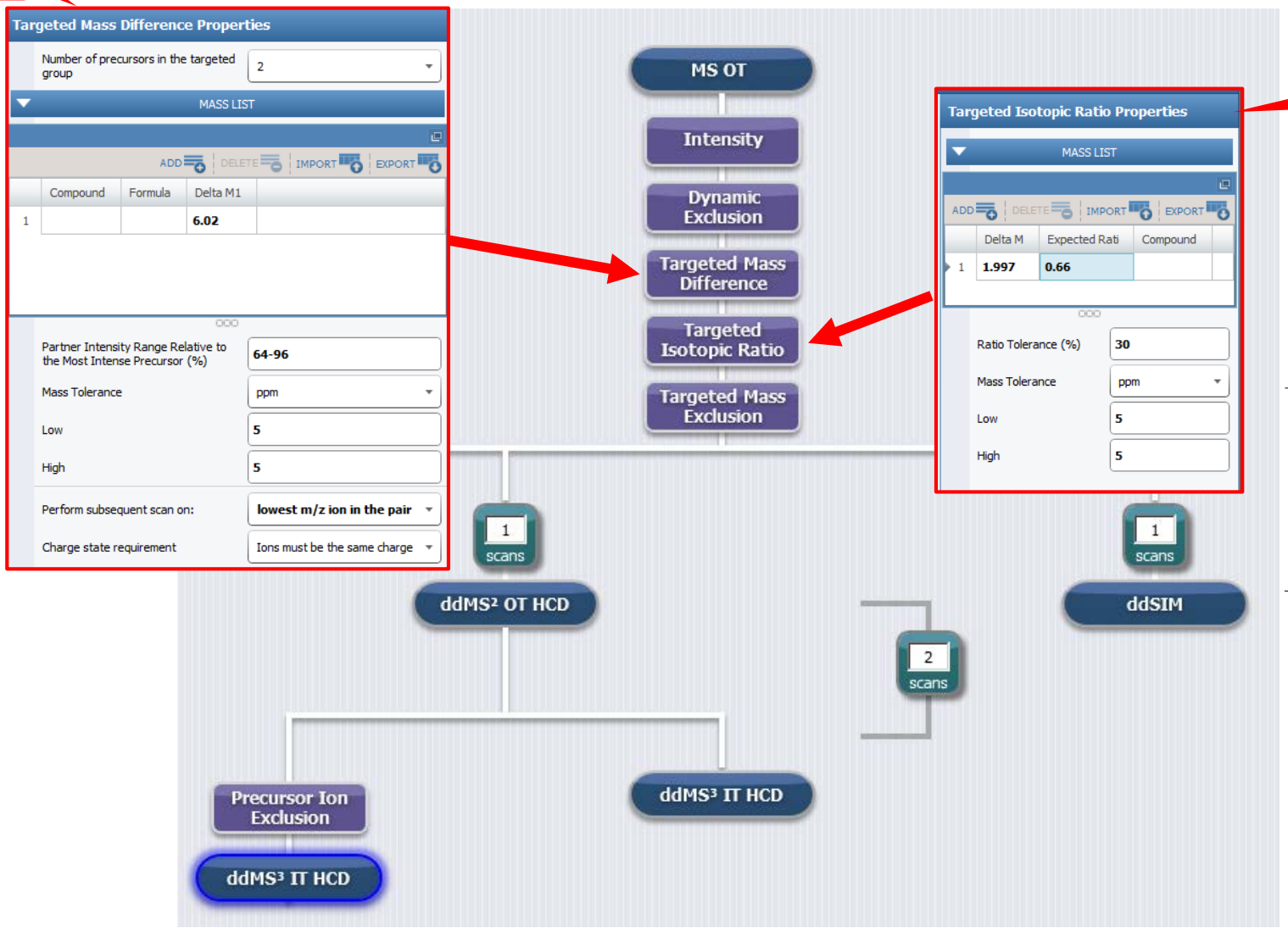
Isotope triggering

- By delta mass: Sulfur, Chlorine
- By isotope peak relative intensity
- By customer-defined mass difference

Comprehensive, feature-specific filters to trigger low abundant components MS^n

Feature Specific Filters Capture Ions of Interest for MS^n

Targeted Mass Difference



Targeted Isotopic Ratio

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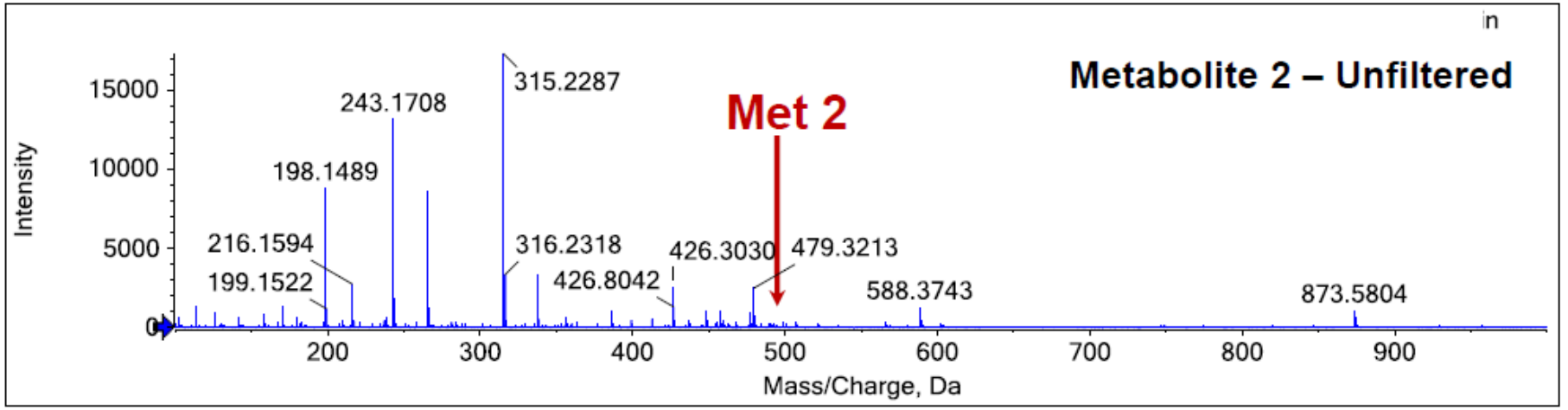
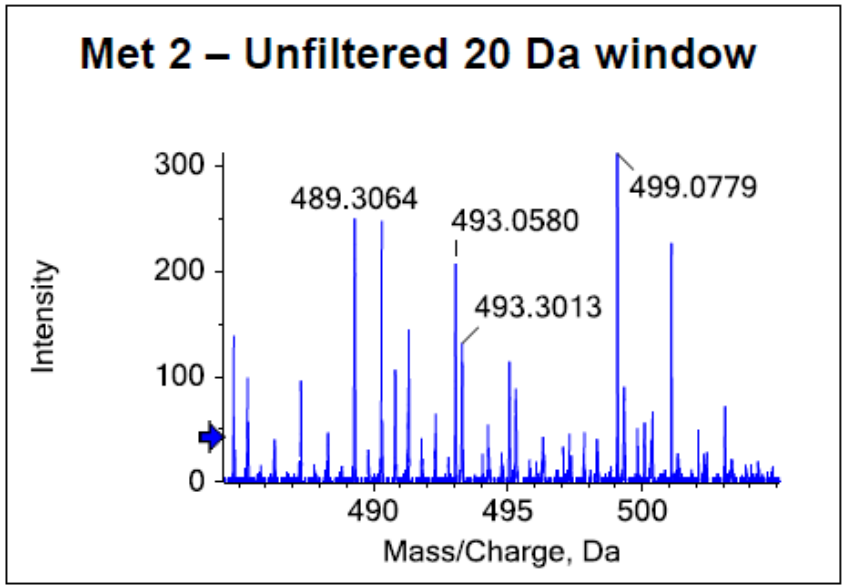
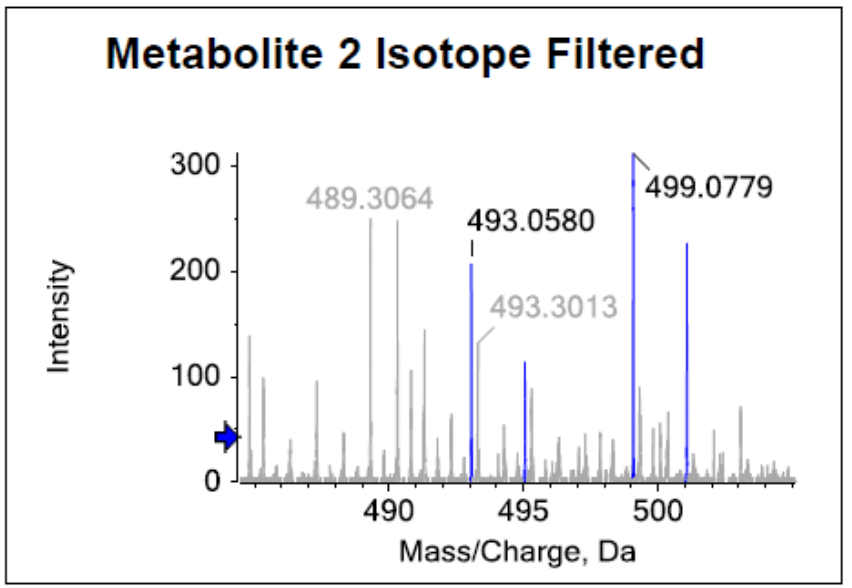
Isotopic Filtering of Mass Spectra



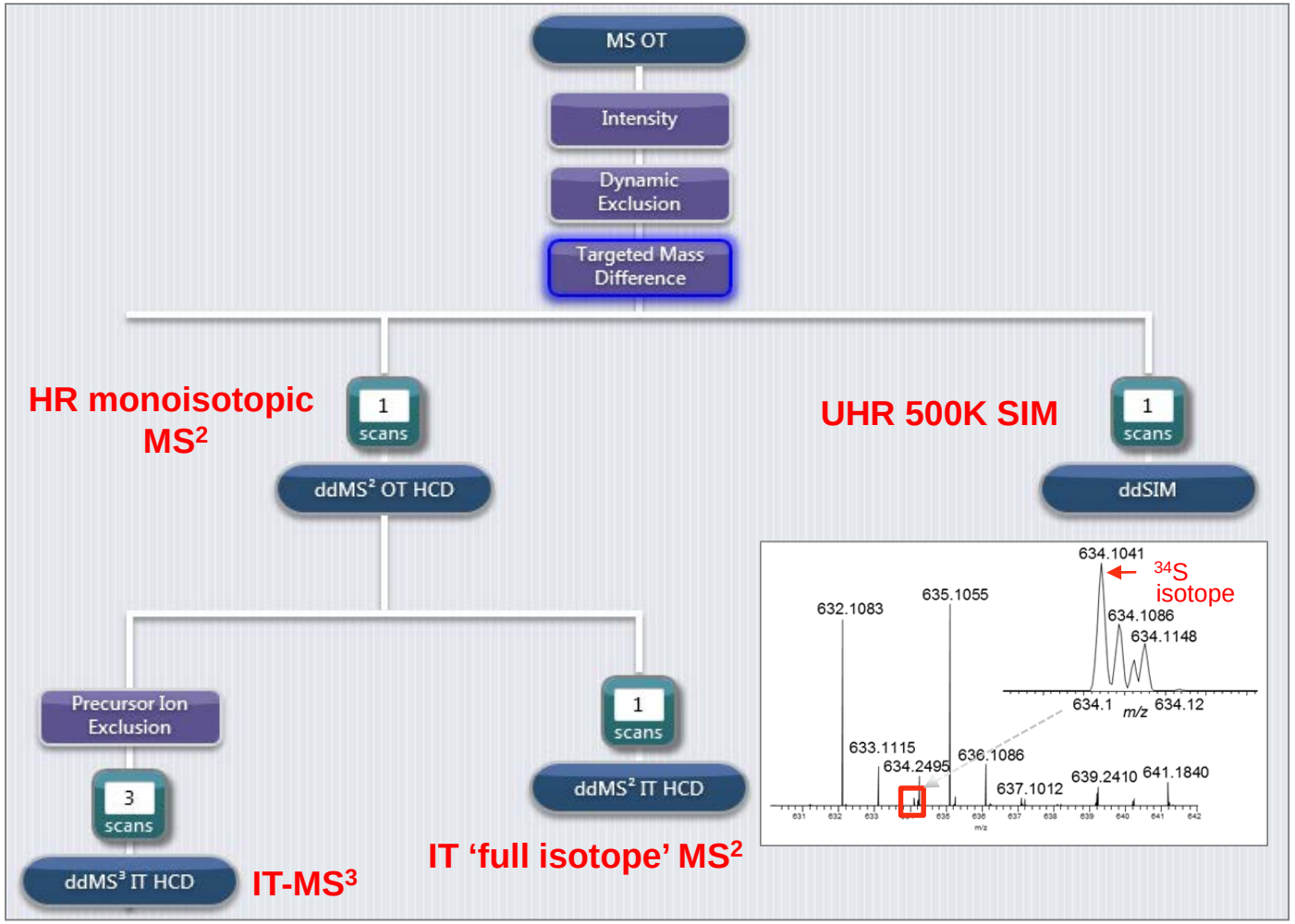




ThermoFisher SCIENTIFIC

Xenobiotics: Labeled Metabolite Identification in Complex Matrices

Ultra High Resolution, High Specificity Experimental Design



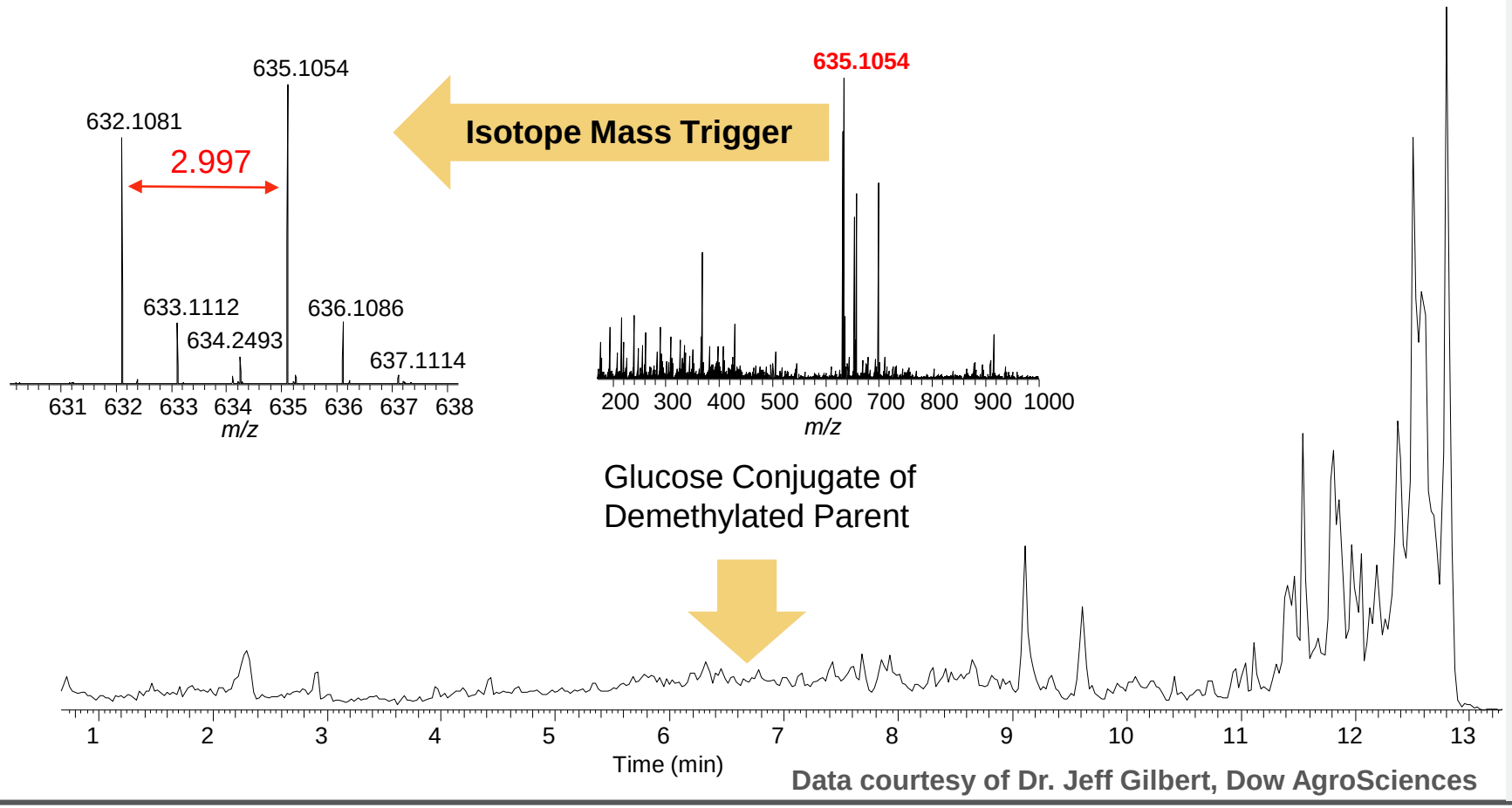
Data courtesy of Dr. Jeff Gilbert, Dow AgroSciences

Structure Elucidation Workflow

- Xenobiotic material is applied as mixture of natural and a stable isotope-labeled material to create an 'un-natural' isotope cluster in resulting metabolites
- 'Mass-Difference' filtered data dependent method acquires OT-MS/MS, (monoisotopic), Ultra High Resolution OT-SIM (Selected Ion Monitoring), IT-MS^3 and IT-MS^2 (full isotope window)
- Unique workflow provides comprehensive spectral data that enables full structural elucidation of unknown metabolites

MS^3 Analysis of Trace Level Components

High Specificity MS^3 Fragmentation of Metabolites

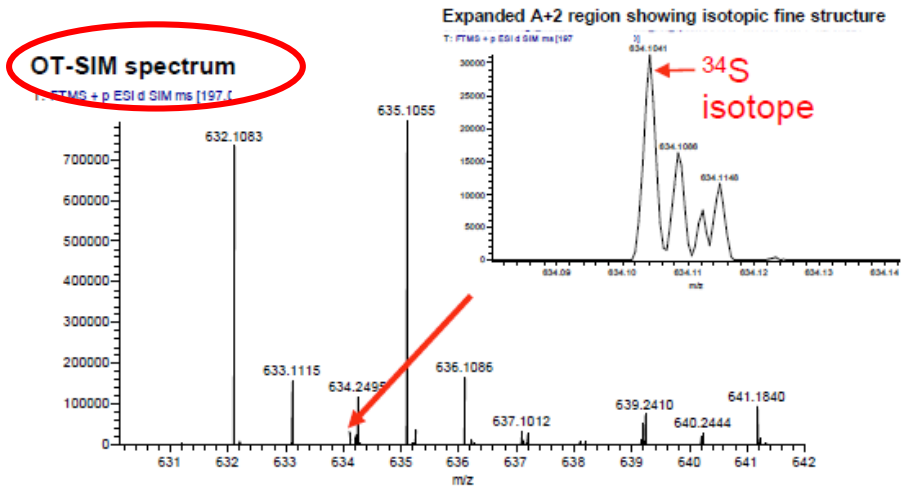


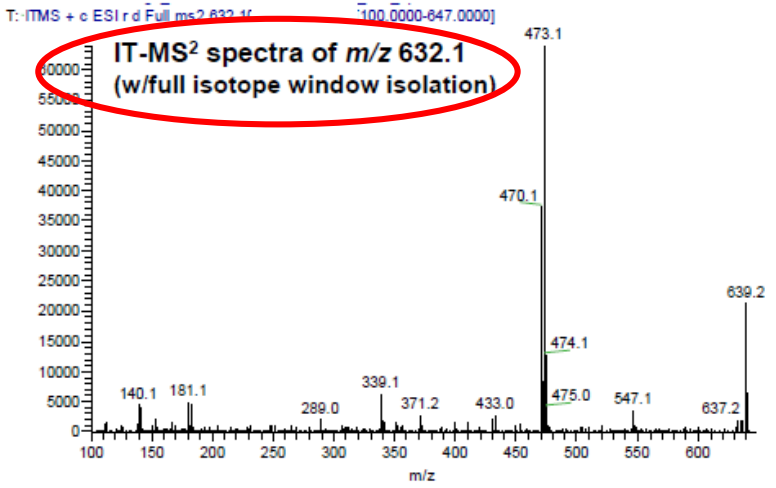
Data courtesy of Dr. Jeff Gilbert, Dow AgroSciences

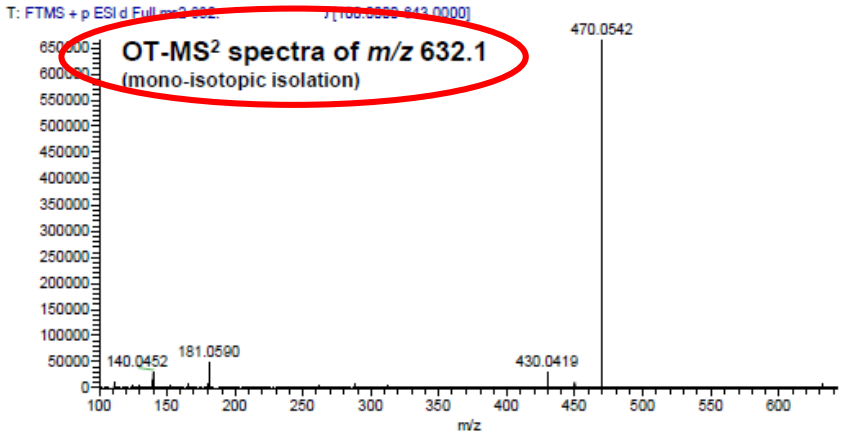
Customized Workflow

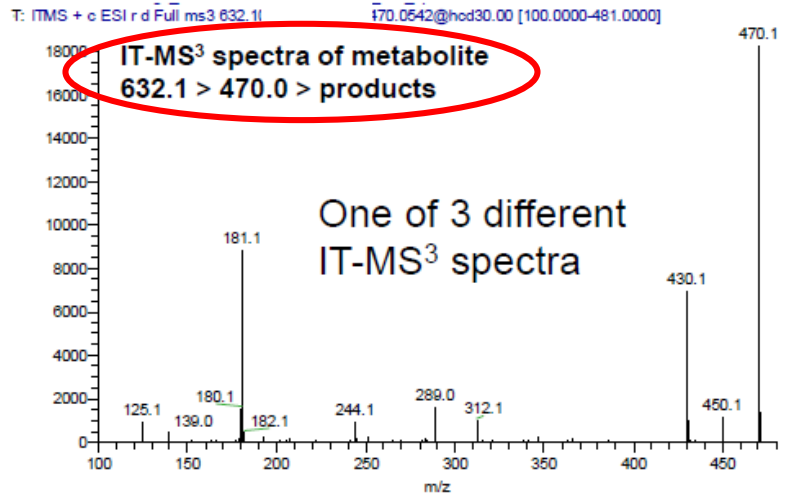
- 'Targeted Mass Difference' filter allows specific triggering of MS/MS scans on precursors that have peaks with a specified mass difference
- High specificity in experimental design allows the detection of trace components with 500K resolution for elemental composition determination
- High sensitivity MS^3 analysis provides fragmentation spectra allowing structure validation
- This highly selective workflow increases throughput by 2X vs. a traditional targeted workflow

Mass Difference Triggered MS^n of Plant Metabolites in Complex Biomatrix









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Orbitrap ID-X MS AcquireX Deep Scan Workflow for MetID and Metabolomics Research

AcquireX Deep Scan Workflow

BACKGROUND EXCLUSION

Create and use an exclusion list of constant background ions and peaks to reduce background fragmentation in your ID runs

What Xcalibur Does:

- Generates up to 1 exclusion list per sequence
- Updates the ddMSn method with the exclusion list
- Automatically inject ID samples with updated ddMSn method

SELECT

EXCLUSION & COMPONENT INCLUSION

Combines exclusion and inclusion lists to automatically and reliably acquire more relevant MSn data in a single injection

What Xcalibur Does:

- Generates up to 1 exclusion list per sequence
- Generates 1 inclusion list per ID injection block
- Injects user-defined number of ID injection blocks

SELECT

DEEP SCAN

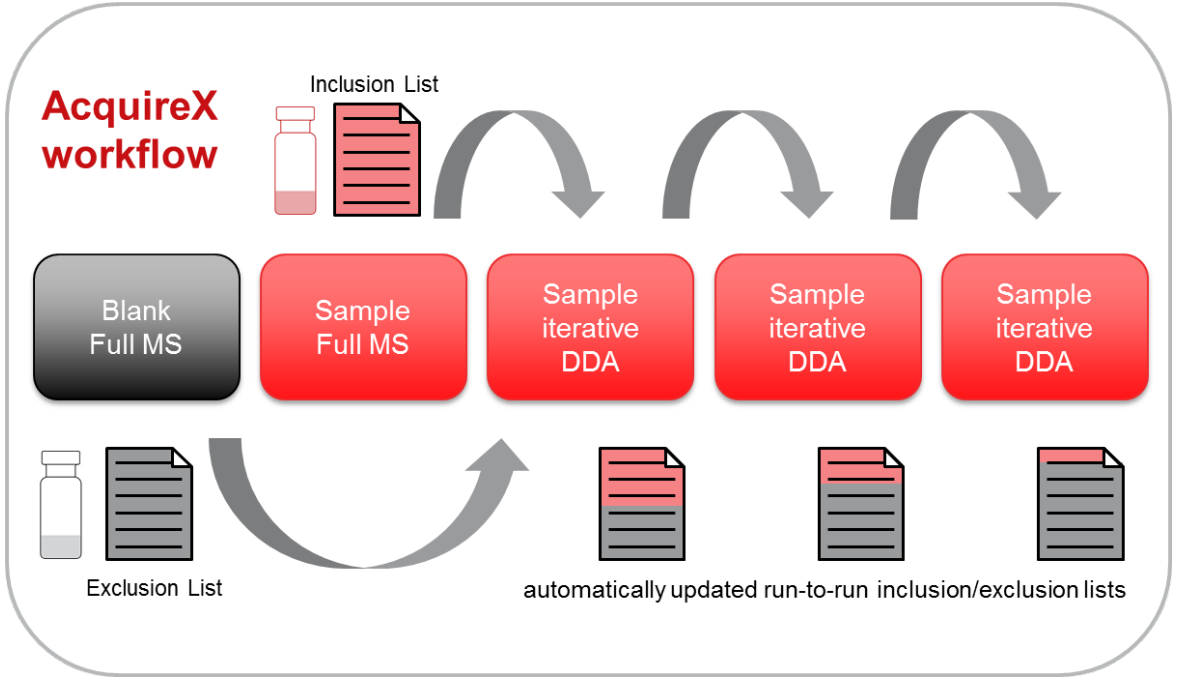
Combines a single exclusion and inclusion list with multiple ID injections to comprehensively fragment relevant precursor ions

What Xcalibur Does:

- Generates up to 1 exclusion list per sequence
- Generates up to 1 inclusion list per sequence
- Injects ID samples until all inclusion list ions are fragmented or user defined number of ID samples are reached

SELECT

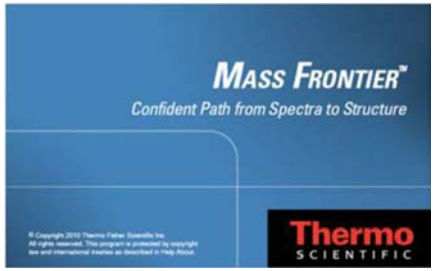
Collect more meaningful data, not just more data to maximize productivity



Compared with Traditional DDA, AcquireX Deep Scan Workflow Significantly Increases ID by Triggering More MS/MS and MS^n

Compound Discoverer 3.0 for Data Processing

Data Analysis Software, Database and Spectral Library Suite



Mass Frontier 8.0
spectral interpretation software

Compound Discoverer 3.0
software

mzVault 2.0
Library Search and Manager

HighChem LLC

Thermo Scientific™TraceFinder™ software
targeted screening and Quan.

Thermo E&L Compound
Database

ThermoFisher
SCIENTIFIC

Thermo Scientific Compound Discoverer Software (CD 3.0)

https://mycompounddiscoverer.com/

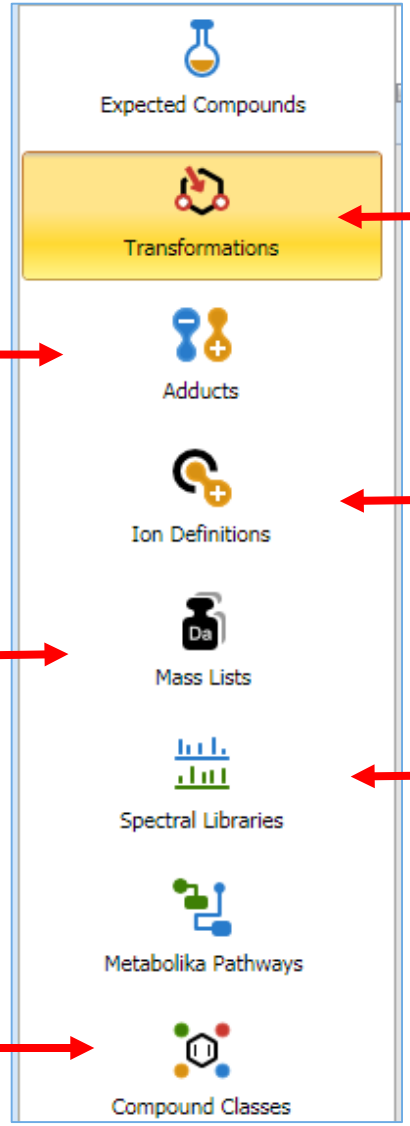
- Component extraction based on HRAM and isotope pattern
- Elemental composition prediction
- Customizable feature set for advanced data processing
- Searching online/offline spectral libraries and multiple databases for ID
- Statistics and differential analysis

Complete Small Molecule Structure Analysis Platform

ThermoFisher SCIENTIFIC

Customizable Feature Set for Data Processing

- Default lists are available.
- All lists can be customizable to tailor fit your application.
  e.g., mzVault spectral library, E&L mass list



Expected biotransformation list

Define adducts based on your experiments

Define ions based on your experiments

Add searchable mass list tailored to your application

Add searchable spectral libraries tailored to your application, e.g., mzVault

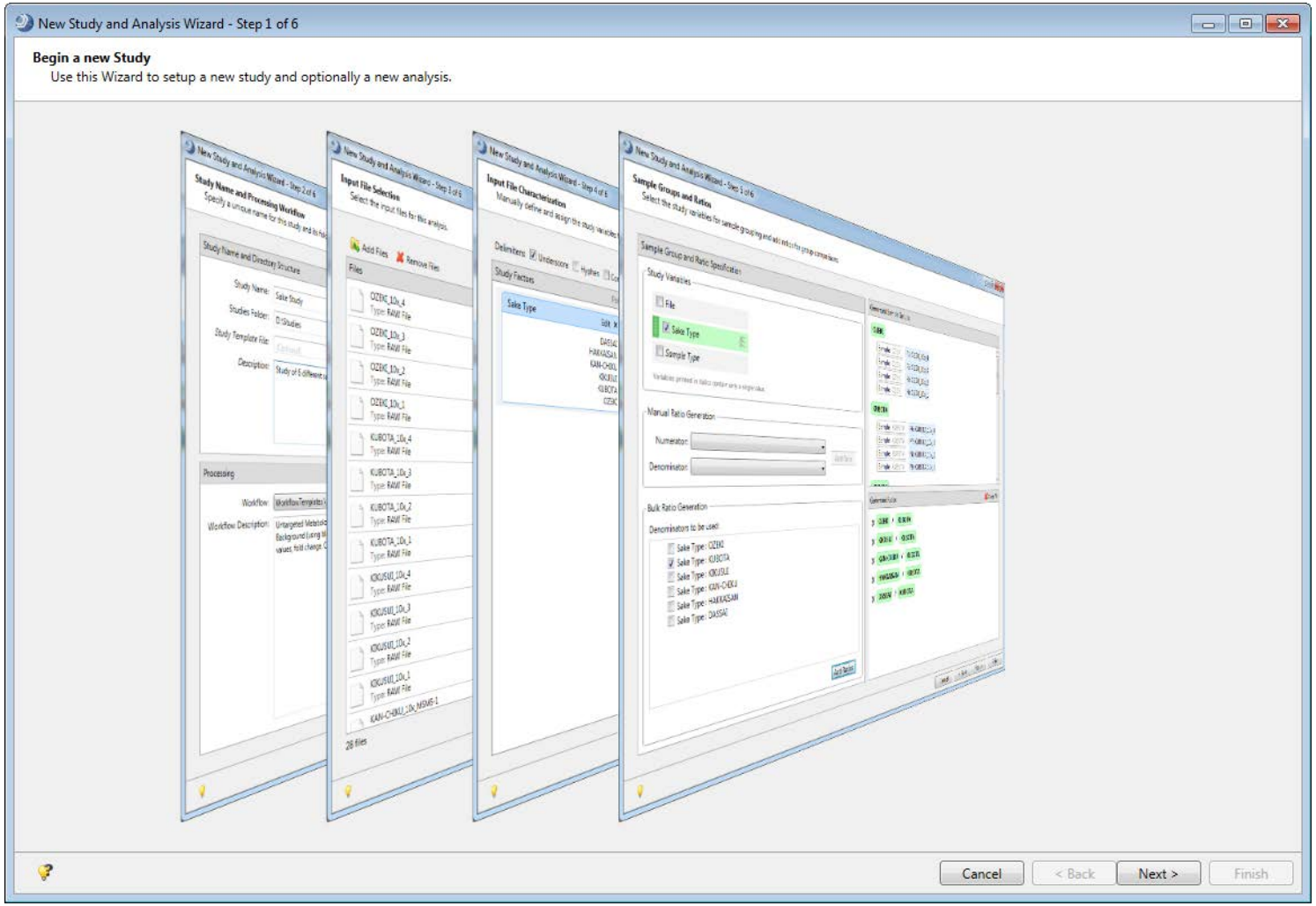
Ions belong to the same compound class e.g., parent fragments

Fragment Ion Search - FISh Algorithm

- Fragment Ion Search – A means of detecting related components in complex samples
- Data is scanned using fragmentation knowledge from a known structure
- Components that share common fragments are detected
- Fragments can be modified (metabolically or otherwise) and still be detected

The Wizard Allows You to Build Processing Workflow

Compound Discoverer 3.0 Software



Description for each step

Creating a study and analysis use the guided “New Study and Analysis wizard” and build-in workflow templates

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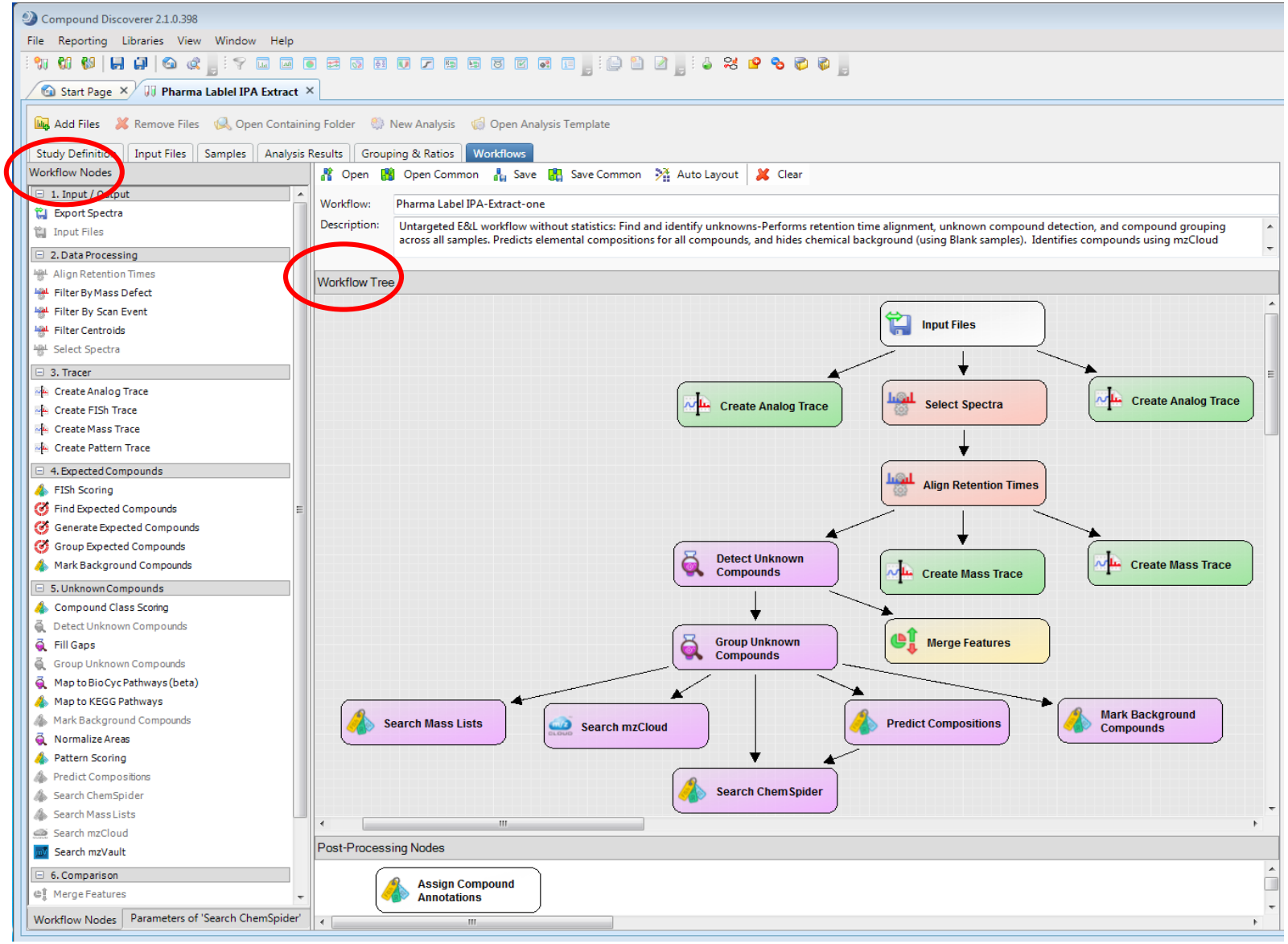
Node-based Processing Workflow



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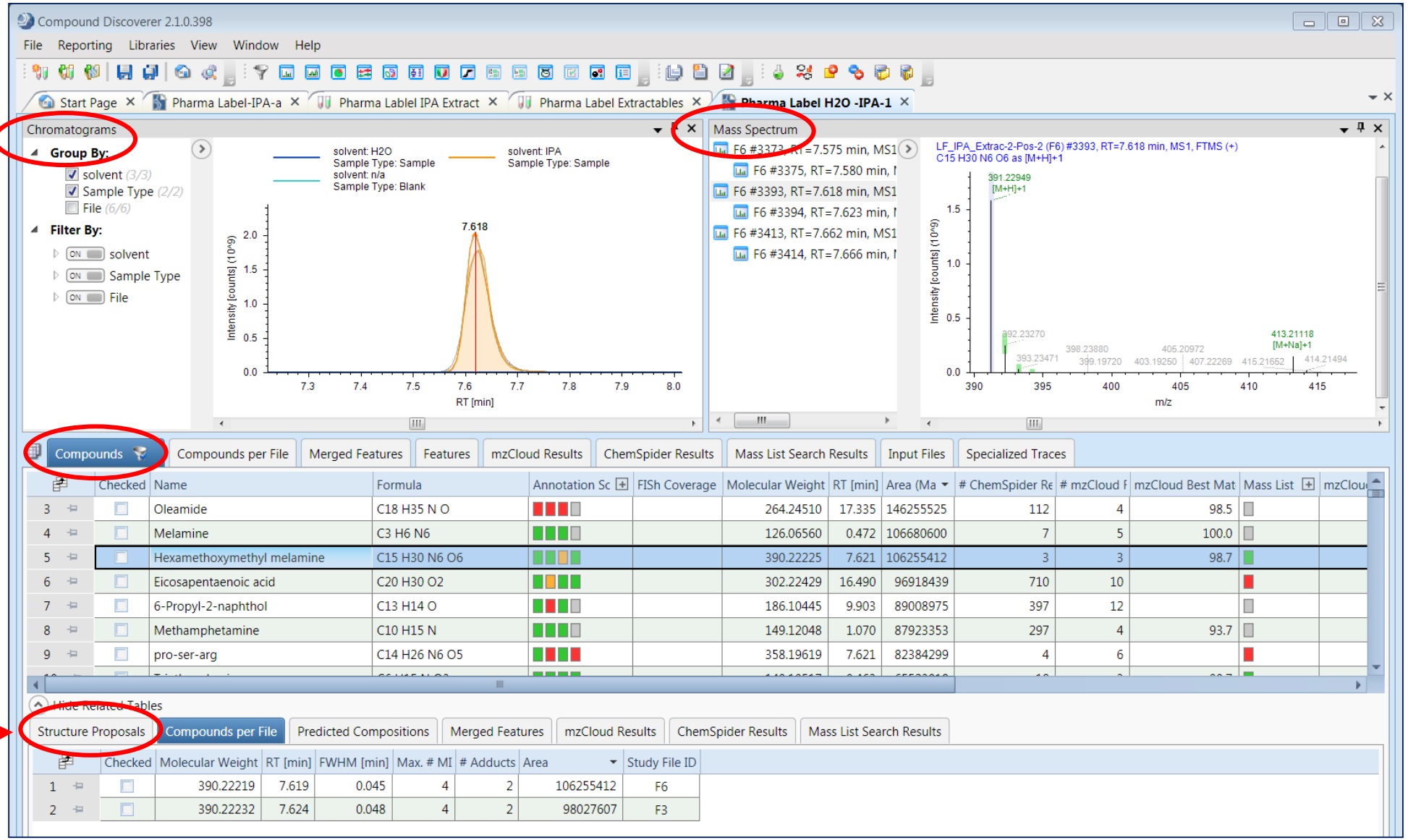
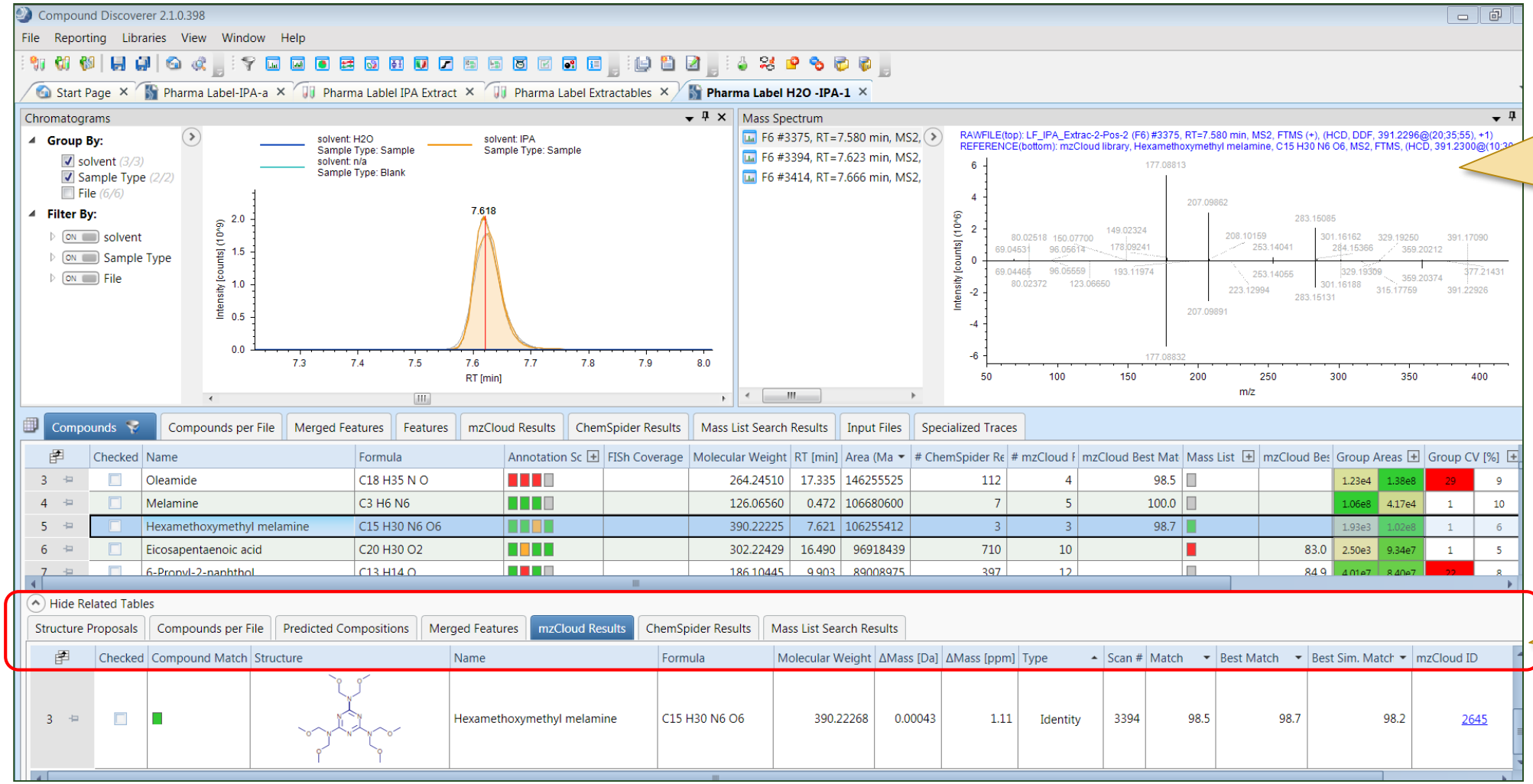
Result View – Data Interpretation



sub-tables

Multiple Database Searching in Parallel to Identify Known Unknowns



Mirror plot for each mzVault hit: observed MS2 vs. library MS2
• Matches collision energy between raw file MS/MS and reference MS/MS

Sub table show detailed info for compound

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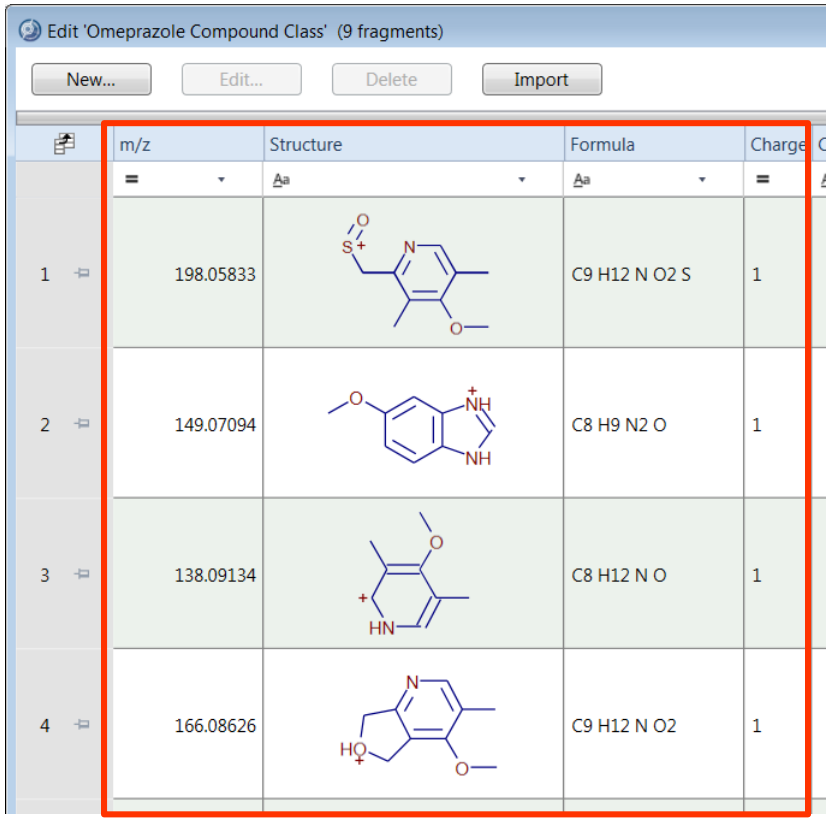
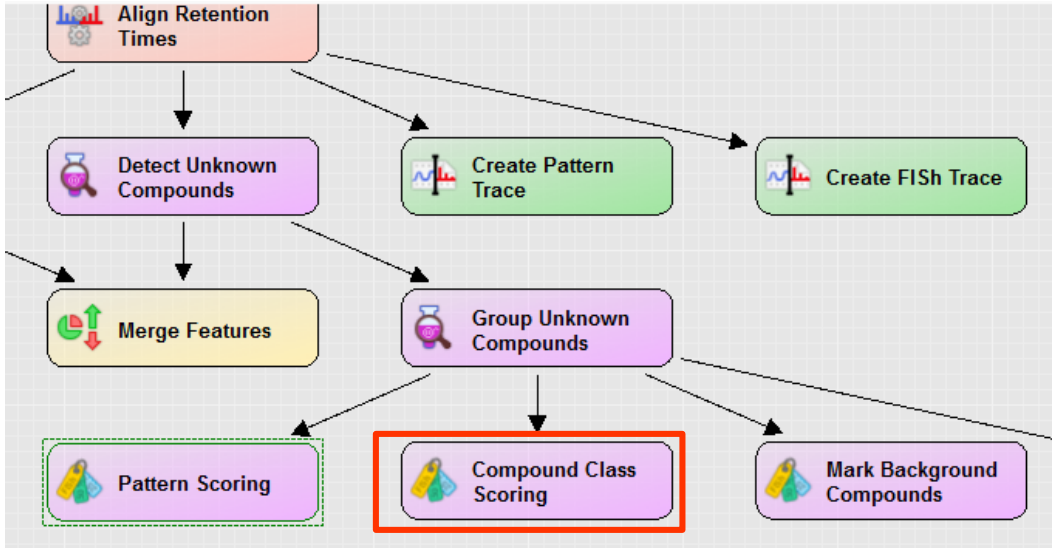
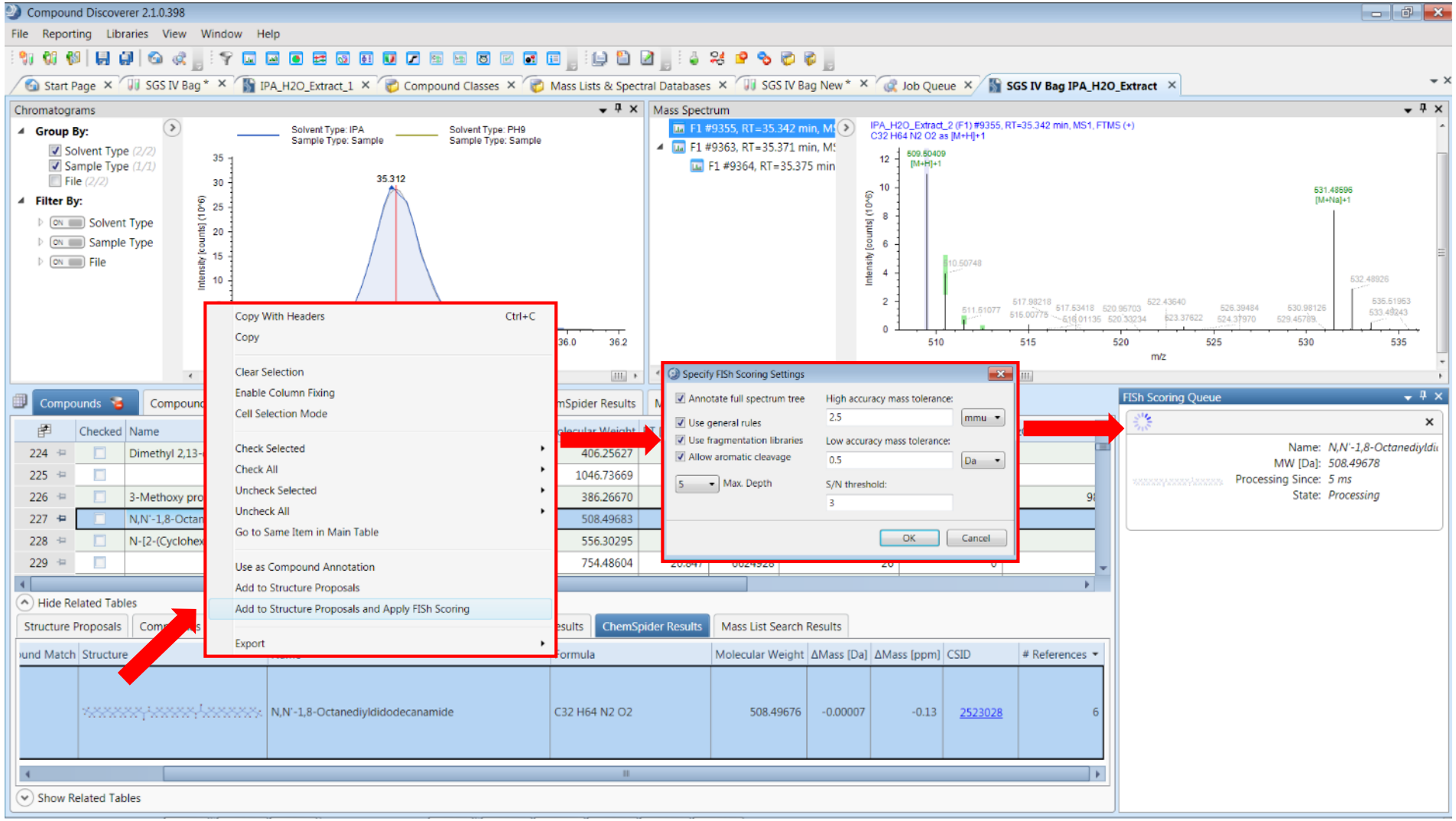
Compound Class Scoring: Tool to Find Structurally Related Compounds





- Create compound class using common fragments from parent and known metabolites
- Use Mass Frontier™ to predict fragments from parent mol file. Copy and paste the fragment structures to construct compound class.

“Structure Proposal & Apply FISh” for Unknown ID



FISh stands for “Fragment Ion Search”

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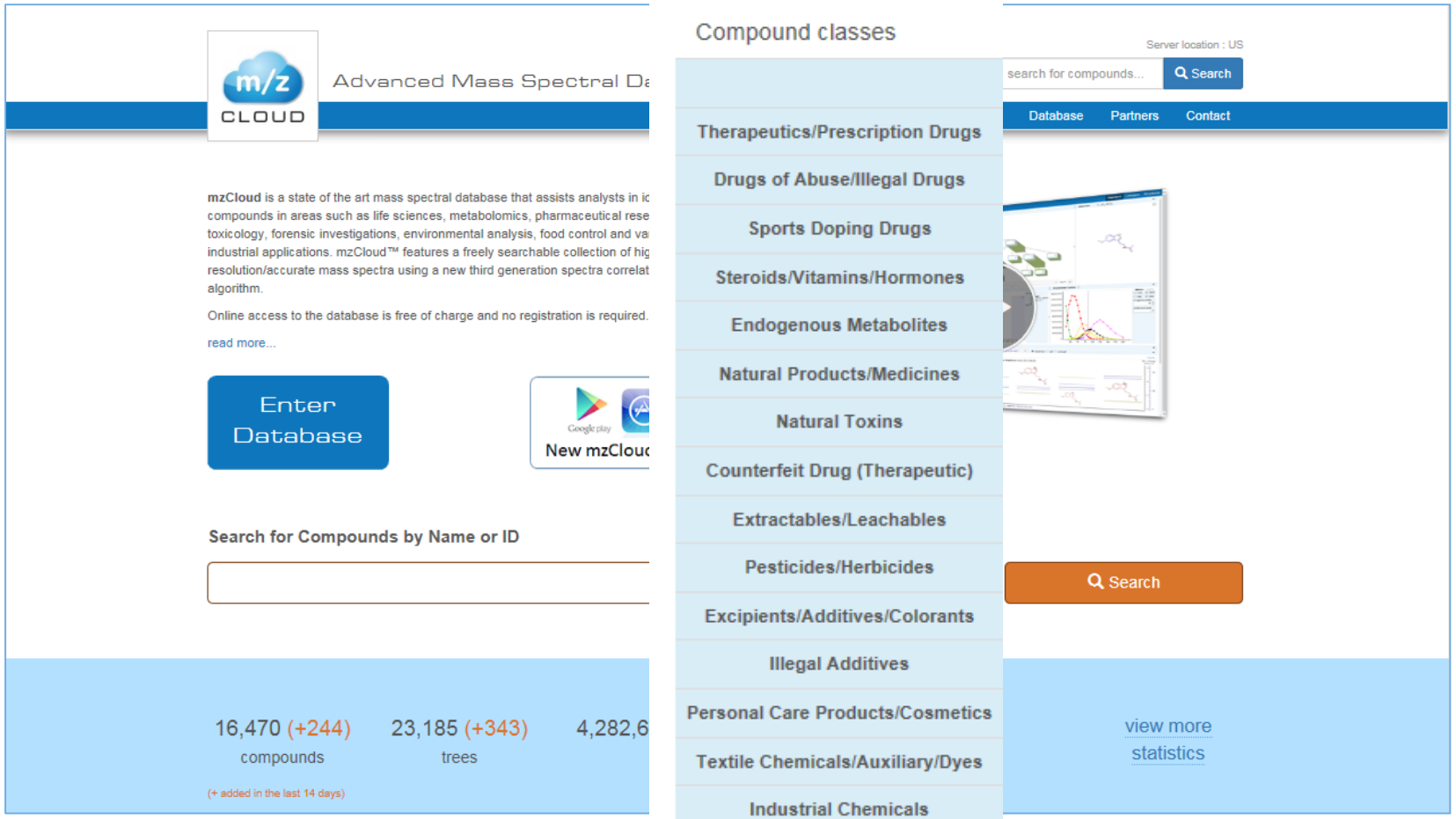
mzCloud - Advanced High Resolution Mass Spectral Database

mzCloud is a trademark of HighChem LLC, Slovakia



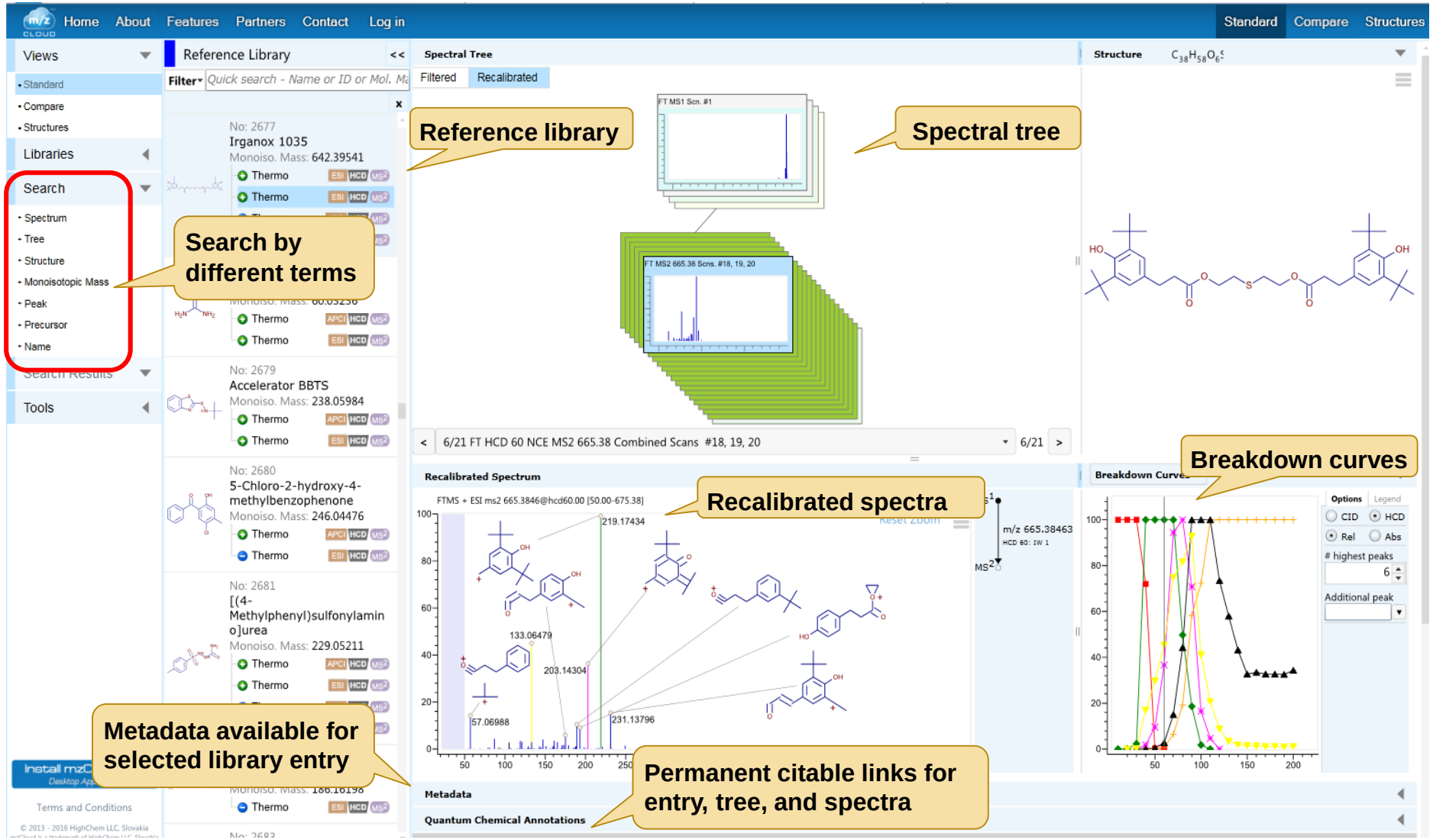
https://w

Free, Available to everyone, everywhere

ThermoFisher SCIENTIFIC

mzCloud Spectral Library

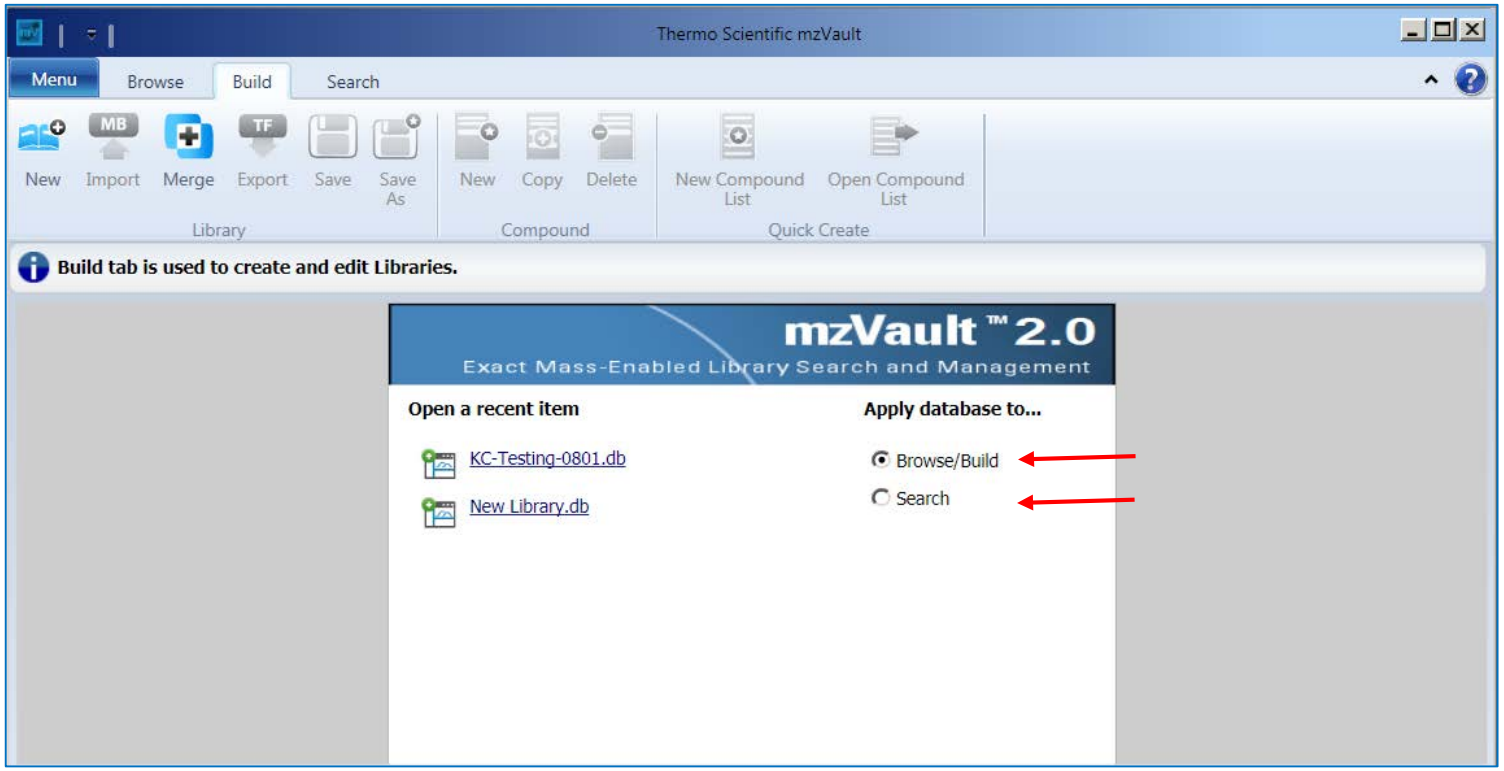
mzCloud is a trademark of HighChem LLC, Slovakia



ThermoFisher SCIENTIFIC

Thermo Scientific mzVault 2.0

Exact Mass-Enabled Library Search and Management
- Searching library – local mzCloud
- Creating customer library



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Summary

- High resolution mass spectrometry is the necessary tool for drug metabolite identification. Only Orbitrap MS delivers high quality HRAM data with high mass accuracy, isotopic pattern fidelity, and MS^n for confident small molecule structure characterization.
- Orbitrap ID-X MS AcquireX data acquisition workflows generate background exclusion lists and inclusion lists automatically, which minimizes matrix interferences and increase triggering MS^n of drug-related ions. AcquireX improves overall metabolite ID and small molecule structure analysis efficiency, quality, and accuracy!
- Compound Discoverer 3.0, Mass Frontier 8.0, and mzCloud suite facilitate confident small molecule structure analyses.

Thermo Scientific Orbitrap ID-X MS for Natural Product Research and Traditional Medicine

thermoscientific

Analysis of Chinese, Korean, and American Ginseng Roots by LC-MS² and LC-MS³ by Orbitrap ID-X

Stephanie Serres, Kate Comstock, Caroline Ding, Ralf Tautenhahn, Seema Sharma, Thermo Fisher Scientific, 355 River Oaks Pkwy, San Jose, CA, USA, 95134

ABSTRACT

Purpose: Interrogate the differences between ginseng roots of varying quality and variety by LC-MS² and LC-MS³. Advanced annotation tools available in Compound Discoverer 3.0 are highlighted including the newly released mzLogic algorithm for ranking candidate structures.

Methods: Ginseng roots of varying quality and variety from Chinese, Korean, and American origin were purchased from a TCM pharmacopeia and were interrogated by LC-MS² and LC-MS³. Samples were finely ground by mortar and pestle followed by an 80% methanol extraction. Samples were chromatographically separated by a 32 minute reverse phase gradient using a Thermo Scientific™ Accucore C18 2.1x 100 mm, 2.6 µm column and then analyzed on the newly released Thermo Scientific™ Orbitrap™ ID-X Tribrid Mass Spectrometer. Samples were analyzed by Full MS² in triplicate and a pooled sample was analyzed by Acquirx® with four iterative data dependent MS² injections for increased compound detection and annotation. Annotations were prioritized based on level of confidence (highest priority being a mzCloud match and lowest being annotation made with Fragment Ion search (FISh)).

Results: Chinese (Jilin) ginseng and both Korean ginseng (high and lower quality) clustered in the principal component (PCA) plot suggesting no major differences between these types of ginseng. Differences were observed with the Chinese (white) and American ginseng. A total of 11,700 compounds had an MS² associated with it. Of those with an MS² associated with the compound 3,000 had either a spectral or exact match to species present in mzCloud. For results that had neither an exact or similar match, mzLogic was applied to rank candidate structures found in the Nitta Terrestrial list or ChemSpider database. The highest ranked candidate structures were analyzed by FISh to explain fragmentation data.

INTRODUCTION

Traditional Chinese Medicine (TCM) has been in practice for thousands of years, incorporating various herbs or roots as form of medical treatment. Alternative to Western medicine, TCM incorporates a holistic approach that is not well understood or accepted by Western medicine. One such root incorporated into many TCM treatments is ginseng, originating from the genus Panax. This root can be cultivated in the wild or purchased from holistic store fronts or TCM pharmacopeias at a reliable cost depending on the origin, variety, and quality of the root.

MATERIALS AND METHODS

Sample Preparation: Ginseng samples were purchased from a local Chinese pharmacopeia and were finely ground by mortar and pestle. 2 mL of 80 % methanol was added to 250 mg of ground ginseng samples and were vortexed for one minute, followed by 1 hour of sonication, vortexed for one minute, and then 1 mL was transferred to a 1.5 mL Eppendorf tube. Samples were then centrifuged at 10,000 rpm for 5 minutes and then transferred to a LC auto sampler vial for analysis. A pooled sample was prepared with equal aliquots added from ginseng #1 through 5 for a separate LC-MS² for the Acquirx® experiments.

Table 1. Ginseng Samples Analyzed. Samples of different origin, variety and quality were purchased from a local Chinese pharmacopeia. Samples #1 through 5 were run in triplicate by Full MS² only, while the pool was analyzed by Acquirx®.

	Origin (quality or variety)	Mass Spec Method
Ginseng # 1	American	Full MS²
Ginseng # 2	Korean	Full MS²
Ginseng # 3	Korean (high quality)	Full MS²
Ginseng # 4	Chinese (Jilin)	Full MS²
Ginseng # 5	Chinese (white)	Full MS²
Ginseng Pool	Mix of # 1 through 5	Acquirx® MS² Deep Scan

Figure 1. Ginseng Samples ground by mortar and pestle. Samples were finely ground by mortar and pestle before they were extracted.

Liquid Chromatography Method:

System:	Vanquish™ Horizon UHPLC
Column:	Accucore C18 2.1 x 100 mm, 2.6 µm
Mobile Phase:	A: Water, 0.1 % Formic Acid B: Acetonitrile, 0.1 % Formic Acid
Column Temp.:	45 °C
Auto Sampler Temp.:	4 °C
Injection Volume:	5 µL
Gradient:	Figure 2

Figure 2. Liquid Chromatography Gradient

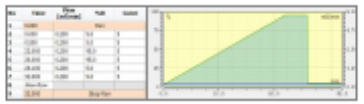


Table 2. HESI Source Parameters. Source conditions used for analysis at LC flow rate of 200 µL/min.

HESI Source parameters			
Polarity	Positive	Sheath Gas	45
Spray Voltage	3.4 kV	Aux/Sweep Gas	10
Capillary Temperature	300 °C	Sweep Gas	1
S-Lens RF	45	Aux/Sweep Gas Temperature	350 °C

Mass Spectrometry Method:

Table 3. Mass Spec Method Parameters

	MS1	MS2	MS3
m/z Range	115 - 1700	100-1700	100-1700
Resolution	60K	30K	15K
AGC	2e5	5e4	1e4
Max Injection Time (ms)	50	80	35
Intensity Range	n/a	1e03 to 1e20	1e03 to 1e20
Dynamic Exclusion	n/a	3 sec	3 sec
HCD %	n/a	25, 30, 50	30

Figure 3. Mass Spec Method Decision Tree (Acquirx®)

Figure 4. Acquirx® Data Acquisition Workflow Employed on Orbitrap ID-X. The inclusion and exclusion lists are automatically built and implemented for increased depth of coverage.

RESULTS

Statistical Differences:

Figure 5. Principle Component Analysis. Method reproducibility demonstrated between triplicate injections and clustering between ginseng samples.

Figure 6. Volcano Plot of American vs. Korean. Compounds of interest highlighted for quick reference in the result table.

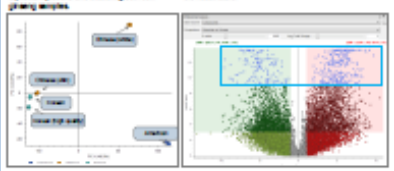
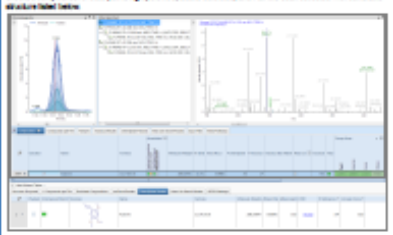


Figure 7. Investigating the Statistically Significant Compounds in the Results Table. Quick reference to identified compounds reveals (left to right) the EIC, scans collected, and Full MS² scan collected with candidate structure listed below.



Compound Annotation Strategies (in Order of Confidence):

1. mzCloud Search – direct match to spectral library (> 9k Compounds, > 2M spectra). The highest confidence annotation are those that have an exact spectral match to compounds in the mzCloud mass spectral library as seen in Figure 8.

Figure 8. mzCloud match to Caryophyllene oxide.

2. mzCloud Similarity Search – similar match to mzCloud (> 9k Compounds, > 2M spectra). If no exact match can be obtained from the spectra available in mzCloud the next most informative information available would be a similarity match to species in mzCloud. This means the experimental spectra obtained does not exactly a known compound in mzCloud but many of the fragments observed are similar and can thus be used to aid in structural elucidation.

Figure 9. mzCloud Similarity match in Chromeleon 7.2.

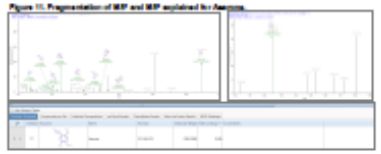
3. Flavonoid Mass List Search With mzLogic Applied (8,548 Flavonoids with structures). This list was obtained from the Nitta Lab as a collaboration. The list contains structures which enables an mzLogic search to rank candidate structures. mzLogic is performed by correlating the fragmentation data and compound structure of known compounds in mzCloud to that of candidate structures.

Figure 10. Match with LeafSpin. Precursor and molecular formula match with flavonoid mass list.

4. ChemSpider Database Search With mzLogic Applied (online database). A precursor and molecular formula search of ChemSpider was conducted in addition candidate structures were ranked with mzLogic. No example provided. See figure 10 for similar result seen with another mass list or database search.

5. FISh and Utilization of MS³. Candidate structures submitted to FISh yielded structural information of the fragments observed based on 500 known chemical reaction mechanisms.

Figure 11. Fragmentation of MS² and MS³ explained for Asarone.



Summary of mzCloud Exact and Similar Matches:

Figure 12. Total of Annotations Assigned to List of Compounds. Of the 8,774 compounds detected 3671 had an mzCloud match a 65 and 3,000 had either an exact or similar match a 50. A total of 5,600 did not have an annotation.

Figure 13. Number of MS² Collected. Of the 8,774 compounds detected 8,2674 had an associated MS², whereas 6,481 compounds did not.

CONCLUSIONS

- Acquirx® enabled on Thermo Orbitrap ID-X Tribrid Mass Spectrometer improved data acquisition for untargeted analysis with unknown annotation.
  - Utilization of Acquirx® allowed for 3,700 compounds with MS² gathered out of 11,700 compounds in the 32 minute method.
  - Of the 3,700 compounds that had an MS² gathered 50 had an exact match to mzCloud (match >70), whereas 3,700 had an exact or similar match to mzCloud.
- The differences in quality and variety observed between American, Korean, and American ginseng were investigated closely using statistical tools in Compound Discoverer 3.0 to quickly find compounds of interest.
  - No major differences observed with high and low quality ginseng
  - Both Korean (low and high quality) closely resembled Chinese (Jilin) ginseng, whereas Chinese (white) and American displayed distinct differences.
- Additional advanced annotation tools available in Compound Discoverer 3.0 led to more compounds with putative identifications.
  - Compounds were annotated with either a direct or similar match to species in mzCloud.
  - For species that did not have a direct or similar match the mzLogic algorithm was applied to rank candidate structures by correlating experimental spectra gathered to compounds found in mzCloud.

REFERENCES

See P-236 for more information on Acquirx® and how it improves depth of coverage for data dependent MS/MS experiments.

TRADEMARKS/LICENSING

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Thank you!

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Thank You

Please return our survey to receive a drink ticket for our daily networking event where you can continue discussions with our experts!